JOURNAL

OF

HELMINTHOLOGY

Founded by

R. T. LEIPER, C.M.G., M.D., F.R.S.

Emeritus Professor of Helminthology in the University of London

Edited by

J. J. C. BUCKLEY, D.Sc.

William Julien Courtauld Professor of Helminthology
in the University of London (London School of Hygiene and Tropical Medicine)

Vol. XXII, 1947-1948

London School of Hygiene and Tropical Medicine
Keppel Street (Gower Street), London, W.C.1
England

CONTENTS OF VOLUME XXII.

No. 1

		PAGE
1.	Goodey, T. On the stem eelworm, *Anguillulina dispsaci*, attacking oats, onions, field beans, parsnips, rhubarb, and certain weeds	1—12
2.	Hickey, M. D., and Harris, J. R. Progress of the *Diphyllobothrium* Epizootic at Poulaphouca Reservoir, Co. Wicklow, Ireland	13—28
3.	Clapham, P. A. On *Vianella africana* n.sp., *Cheilospirura falconis* n.sp., and *Oesophagostomum tridentatum* Maplestone, 1932	29—36
4.	Clapham, P. A. On the identification of some species of *Trichostrongylus*	37—46
5.	Clapham, P. A. On the occurrence of *Davainea madagascariensis* on the African mainland...	47—48
6.	Young, M. R. The Incidence of *Trichinella spiralis* at Necropsies in England	49—60
	Actual dates of publication of Vols. I—XXI of the Journal of Helminthology	61—62

No. 2

1.	Crusz, H. Observations on a case of Endogenous Budding in *Cysticercus tenuicollis* Rudolphi	63—72
2.	Crusz, H. On an English case of an intramedullary spinal Coenurus in Man, with some remarks on the identity of *Coenurus* spp. infesting Man	73—76
3.	Singh, S. N. Studies on the Helminth Parasites of Birds in Hyderabad State. Nematoda I	77—92
4.	Kung, C. C. Some New Nematodes from the Australian Wallaby (*Macropus rufogrisea fruticus*) with a Note on the Synonymy of the Genera *Zoniolaimus, Labiostrongylus* and *Buccostrongylus*	93—108
5.	Goodey, J. B. The Galls caused by *Anguillulina balsamophila* (Thorne) Goodey on the leaves of *Wyethia amplexicaulis* Nutt. and *Balsamorhiza sagittata* Nutt.	109—116

Nos. 3/4 PAGE

1. Peters, B. G. Potato Root Eelworm, D-D, and Soil Sterilization. I. Methods and Criteria ... ... 117—127
2. Peters, B. G. Potato Root Eelworm, D-D, and Soil Sterilization. II. Results for 1946 ... ... 128—138
3. Goodey, T. A note on the presence of phasmids on the male tail of *Anguillulina multicincta, A. erythrinae* and *A. robusta* ... ... 139—140
4. Kung, C. C. On some new species of Spirurids from Terrestrial Vertebrates, with notes on *Habronema mansioni, Physaloptera paradoxa* and *Hartertia zuluensis* ... 141—164
5. Crusz, H. On the Transverse Fission of *Cysticercus pisiformis* in experimentally infested Rabbits, and the Phylogenetic Significance of Asexual Phenomena in Cysticerci ... ... 165—178
6. Crusz, H. Further Studies on the Development of *Cysticercus fasciolaris* and *Cysticercus pisiformis,* with special reference to the Growth and Sclerotization of the Rostellar Hooks ... ... 179—198
7. Singh, S. N. Studies on the Helminth Parasites of Birds in Hyderabad State. Nematoda II ... ... 199—218
8. Singh, S. N. *Gastronodus strasseni* Singh, 1934, and its affinities with *Spirocerca lupi* (Rudolphi, 1809) Chitwood, 1933 ... ... 219—225

Index ... ... 226—230

Journal of Helminthology, Vol. XXII, No. 1, 1947, *pp.* 1—12.

On the stem eelworm, *Anguillulina dipsaci*, attacking oats, onions, field beans, parsnips, rhubarb, and certain weeds.

By T. Goodey, D.Sc., F.R.S.

(*From the Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St. Albans*).

Introduction.

Observations made by various workers over many years have indicated more or less clearly the probability that there is in this country a race of the stem eelworm, *Anguillulina dipsaci,* capable of attacking oats, field beans, onions, rhubarb, parsnips and certain common weeds. One of the earliest records bearing on this is that provided by Bos (1892) who cited information, supplied to him by Miss Ormerod, which showed that field beans might be seriously attacked by the parasite when grown after oats which had suffered from "tulip-root". Many years later Johnson (1936a & 1940) reported failure of beans from eelworm attack following "tulip-root" oats in fields in Yorkshire and showed the significance of common chickweed and cleavers as reservoir hosts on which the parasite can live in the absence of susceptible farm crops. In another paper (1936) he showed that *A. dipsaci* associated with crown-root of rhubarb is interchangeable between rhubarb and oats in both of which it is capable of setting up disease symptoms. In his 1940 paper this investigator showed that *A. dipsaci* from rhubarb readily infests oats and common chickweed and also transfers to beans and, further, that the parasite from both oats and beans is infective to cleavers and common chickweed and back from both these weeds to oats and beans.

Goodey (1937) showed that the parasite causing "bloat" in onions could readily set up disease in the leaves, leaf-stalks and crown of parsnips and at least one later record has confirmed this transference on a field scale. With regard to *A. dipsaci* attacking rhubarb the writer effected its transference, early in 1946, to onions with the production of typical "bloat" symptoms. Observations and experiments carried out by the writer in recent years on "tulip-root" of oats and "bloat" of onions, coupled with the finding of the parasite in certain common weeds amongst affected crops of oats and onions, together with

the results of numbers of cross-infection experiments all lend support to the view that there is a single race of the parasite capable of attacking all these host plants. The purpose of the present paper is to bring together the relevant data which support this conclusion.

Direct Transference: Oat to Onion and Vice Versa.

In an experiment carried out in 1934 to test the range of host plants susceptible to the eelworm causing "tulip-root" in oats, one of the plants selected was onion. Dried diseased oat material, used as an inoculum, was distributed on the surface of the soil in a 3-in. pot. It was covered with a thin layer of soil and on this the onion seed was sown and covered with a further layer of soil. Some of the onion seedlings which grew showed typical symptoms of "bloat" and on dissecting one or two of them numerous adults of *A. dipsaci,* as well as eggs and larvae, were found in the swollen tissues. This was clear evidence that the eelworm could transfer direct from oat to onion and give rise to typical "bloat" disease.

Evidence of transfer in the opposite direction from onion to oat was obtained experimentally several years later. On a small plot at this Institute on which the onion race had become well established, oats of the variety Golden Rain (known to be a susceptible variety) were sown in the spring of 1946 and in several of the resulting plants mild but typical symptoms of "tulip-root" were found. Dissection of the swollen bases of one or two such plants revealed the presence of adults, eggs and larvae of *A. dipsaci,* thus showing the ability of the parasite to transfer from onion to oat.

The writer is also able to adduce evidence of a similar character which is provided by a plot on the trial grounds at the National Institute of Agricultural Botany, Cambridge. In 1943 onions were grown on an area where they had not grown for at least 15 years but where, during the previous 5-6 years, the ground had been cropped with oats which had suffered from eelworm attack. Some considerable areas of the onions suffered from "bloat" and large numbers of them were destroyed. There seems to be no reason to doubt that the eelworm causing disease in oats was responsible for the attack on the onions. In 1944 oats, in several varieties, were again grown on this land and some of the plants showed evidence of damage from "tulip-root". This land apparently harbours a race of the stem eelworm which is interchangeable between oats and onions.

Weeds as Hosts.

Observations made in recent years by a few workers have shown that certain weeds of common occurrence on arable fields and garden soils can serve as hosts of the race of *A. dipsaci* attacking oats and field beans. Johnson (1936a and 1940) found in a number of fields in Yorkshire, where oats were badly attacked by the parasite, specimens of common chickweed (*Stellaria media* Cyrill.) and cleavers (*Galium Aparine* L.) infested by it. Cleavers was also found to be infested in other fields where field beans were severely affected by *A. dipsaci*. It is clear from his observations that the eelworm can persist on these two weeds in the absence of oats or beans and that, most probably, the race affecting oats and beans is one and the same. Staniland (1945) reported the finding of *A. dipsaci* infestation on certain weeds in Devon fields in which oats were attacked by the parasite. In one field common chickweed (*Stellaria media* Cyrill.), field mouse-ear chickweed (*Cerastium arvense* L.) and cleavers (*Galium Aparine* L.) were infected. In another field a little common chickweed and an occasional plant of field mouse-ear chickweed were infected whilst in a third field thyme-leaved sandwort (*Arenaria serpyllifolia* L.) was infected in addition to cleavers, common chickweed and field mouse-ear chickweed. As a result of cross-infection experiments, in which infected weeds were used as inocula, Staniland was able to show that the strain of *A. dipsaci* involved was interchangeable between common chickweed and oats, that infecting cleavers and thyme-leaved sandwort was transmissible to oats but infection in the reverse direction was not affected experimentally. Staniland tentatively suggested that his observations pointed to the possibility of a strain interchangeable between all five host plants namely, common chickweed, cleavers, field mouse-ear chickweed, thyme-leaved sandwort and oats but considered that further experiments were necessary to establish this.

At this Institute the writer has had two special plots under observation for a number of years. One of these has been devoted to the study of "tulip-root" in oats since 1934. By the addition to the soil of dried diseased oat plants, obtained from affected field crops, the infectivity of the soil has been built up and maintained and a large number of oats, both winter and spring varieties, have been tested for susceptibility to eelworm attack. Among the weeds appearing on this plot plants of common chickweed have several times been found infected

with *A. dipsaci.* Cleavers also became infected and showed characteristic signs of attack in the discoloration of the lower leaves when clean seedlings were brought in and planted at one end of the plot. On the other plot, which has been devoted to the study of onion "bloat" a high degree of infectivity has been built up by the addition to the soil of bloated onion bulbs and plants so that when onions are now grown on it there is a heavy mortality of seedlings due to attack from *A. dipsaci.* Common chickweed growing on this plot has often been found to be heavily infested with the parasite and a new centre of infection was set up on a neighbouring plot in 1943 merely by pulling up plants of common chickweed, allowing them to dry a little and then digging them into the surface soil on the new site. On this plot, too, scarlet pimpernel (*Anagallis arvensis* L.) has been found heavily infested with the parasite accompanied by marked stunting and malformation of the foliage. Diseased specimens of this weed were used in 1944 as an inoculum for a cross-infection experiment an account of which is given later on. A single, self-sown plant of cleavers growing on this plot was also found to be infected in the lower discoloured leaves with good numbers of the parasite in the tissues. From these two plots, therefore, there is clear evidence that *A. dipsaci* attacking oats and onions also parasitizes common chickweed and cleavers. The onion race also severely attacks scarlet pimpernel and it will be shown later that this weed is equally well attacked when growing among "tulip-root" oats. A fact worthy of note in passing is that an occasional self-sown plant of red clover when found growing on this plot amongst bloated onions is always healthy in appearance and never reveals eelworm in the tissues; thus pointing to the clover-infecting race of *A. dipsaci* being quite distinct from the oat and onion race.

At the end of June, 1944, the writer paid a visit to a farm in the east of Hertfordshire on two fields of which crops of oats, var. Golden Rain, were showing severe eelworm damage with marked "tulip-root" symptoms. Growing amongst the affected plants in both fields were numerous weeds and among examples which were collected and later examined in the laboratory, infestation with *A. dipsaci,* accompanied by discoloration and/or distortion of the tissues, was established in the following :—

Cleavers (*Galium Aparine* L.). Many young plants showed a tufty, compact and rather twisted appearance of the discoloured leaves close to the soil. In these *A. dipsaci* was plentiful.

Black bindweed (*Polygonum Convolvulus* L.). Several young plants, ranging from 2-5ins. tall, had swollen, distorted nodes and short leaf petioles which were easily broken, and discoloured leaf lesions. *A. dipsaci* in good numbers.

Common chickweed (*Stellaria media* Cyrill.). A number of small plants showing a tufted compact appearance at the centre had *A. dipsaci* in abundance. Examples of this weed were not very plentiful.

Scarlet pimpernel (*Anagallis arvensis* L.). A few plants collected showed small lesions on the leaves and petioles and contained fair numbers of the parasite.

Procumbent speedwell (*Veronica agrestis* L.). One or two plants, when placed in a Baermann funnel and extracted with water, yielded a few examples of *A. dipsaci* but no signs of attack were visible. Some of the above weeds were later used in cross-infection experiments to be described later.

A second visit was paid to the same farm a year later when the same two fields were examined for the presence of weeds infested with the parasite. One of the fields was partly under maize and partly under kale and hoeing and singling of the kale were in progress at the time of the visit with the result that weeds were not plentiful. Nevertheless by careful search a number of young plants of both common chickweed and cleavers were collected which, on later examination, were found to be infested with *A. dipsaci.* The second field had a good stand of barley but again a few plants of common chickweed and cleavers were collected which proved to be lightly infested with the parasite. A third field, which was not seen on the 1944 visit was examined on this occasion. It was carrying a crop of oats, var. Golden Rain, considerable areas of which were seriously damaged by "tulip-root". Young plants of common chickweed and cleavers were collected from amongst the oats and proved to be infested with *A. dipsaci.* Worthy of note in connection with this field is the fact that the oats had been undersown with red clover which, at the time of the visit, was 3-4ins. high and showed, on close examination, no sign of eelworm attack at all; thus indicating that the oat race of the parasite does not produce typical symptoms of eelworm disease on red clover. This is in agreement with the failure of the onion race to attack clover previously noted on p. 4.

Cross-Infection Experiments from Infected Weeds.

1. On 8.ix.1944, the following cross-infection experiment was set up to test the possibility of transference of *A. dipsaci* from infected weeds, collected in June on the farm in east Hertfordshire, to certain other host plants. 20 clean 5-in. flower pots were filled to within 1 inch of the rim with loamy soil which after sieving was mixed with a little silver sand. This soil was known, from previous experiments, to be free from *A. dipsaci*. The pots were arranged in 4 sets of 5 in each and were sown with seeds of the following, one pot to each: oats (var. S. 147); onion (var. Rousham Park Hero); field bean (var. ?); English red clover; mangold (var. Red Intermediate).

Row 1. Uninoculated, to serve as control.

Row 2. Inoculum of dried infested oat material so as to give a comparison between whatever disease symptoms might result from the weeds and that from oats direct.

Row 3. Inoculum of dried infected cleavers (*Galium Aparine* L.).

Row 4. Inoculum of dried infected black bindweed (*Polygonum Convolvulus* L.).

Each inoculum was distributed as evenly as possible over the surface of the soil in the pots and was then covered with about ¼in. of fine soil on which the seeds to be tested were then sown, except the field beans which were planted a little deeper in holes. After covering the seeds with about ½in. of soil and making the surface firm, the pots were watered from below by standing up to the rim in a deep sink of water and they were finally plunged in a bed of ashes situated in the open.

In all the pots the seeds germinated evenly and well and by 11.x.'44 *i.e.* after 33 days, it was clear that whereas in all 5 of the uninoculated pots the seedlings were clean and healthy in appearance, those in the inoculated rows were showing disease symptoms. These were most severe in the case of the 5 pots inoculated with diseased oat material but in the cleavers and black bindweed rows signs of disease were quite apparent though not so marked.

OATS INOCULUM.

Oats. Very severe attack. 6 seedlings which had grown to between ½ and ¾in. in height had been killed by the 33rd day. Three others had grown a little better but were stunted, swollen and discoloured at the bases of the leaves. Adults, larvae and eggs of *A. dipsaci* were abun-

dant in the tissues. In this pot there grew a seedling of annual purple dead-nettle (*Lamium purpureum* L.) which showed brownish-red lesions of the cotyledons and their petioles in which there were found many examples of the parasite.

Onions. Most of the seedlings were killed outright. Three small and very distorted ones were lying on the surface of the soil by the 33rd day. Two of these were teased up in water and fair numbers of the parasite, together with free eggs and larvae were found in the inflated tissues.

Field beans. Three rather stunted seedlings showed twisted leaf-blades with easily visible, scattered brown lesions on the underside. Leaf petioles and stipules were short, swollen and discoloured. The main stem at and just above soil level had extensive lesions and was much discoloured. One of these plants which was fixed in copper acetate-acetic acid to preserve the coloration, when examined under the binocular microscope, showed coiled masses of adult *A. dipsaci* as well as large numbers of free eggs in the vicinity of the scattered brown lesions on the stem, stipules and leaves.

Mangolds. Several of the seedlings showed severe lesions of the stem cortex as well as of the cotyledon petioles and the tissues surrounding the primary shoot. The cotyledons themselves also showed discoloured lesions. In all these situations *A. dipsaci* was plentiful and consisted of many pre-adult and adult forms as well as free eggs which were present along with the adults in the lesions of the cotyledons and it is evident that this race of the parasite can successfully attack and reproduce in mangold tissues.

Red clover. Numerous seedlings showed great damage to the tissues. There were extensive brown lesions on the stem especially in the tissues around the primary shoot, which in many cases was destroyed, in the cotyledons and their petioles. Careful dissection of unstained seedlings and of others which were stained in Scarlet R. showed groups of *A. dipsaci* within the lesions and most of the forms appeared to be pre-adults or young adults. None of the females were gravid and no free eggs could be found in the invaded tissues. This result is in accord with the writer's earlier (1941) findings in which young clover seedlings were seriously injured by the parasite from "tulip-root" oats. Though invasion and destruction of clover seedling tissues takes place the oat race of the parasite fails to establish itself and reproduce in red clover.

CLEAVERS INOCULUM.

Oats. Two seedlings manifesting typical early "tulip-root" symptoms were dug up. They were stunted and the leaf bases were swollen and discoloured. One seedling was stained in Scarlet R. and on subsequent examination, large numbers of adult *A. dipsaci* and numerous free eggs were found in the diseased tissues both of the leaf blades and leaf bases. In this pot also there grew a small tufted seedling of common chickweed which on later examination, after staining in Scarlet R. was found to be quite heavily infected with *A. dipsaci.* This shows that the infection can easily pass from one weed to another.

Onions. Five or six seedlings showed symptoms of "bloat". Two much damaged ones were stained in Scarlet R. and in each, some few adults of *A. dipsaci,* as well as free eggs, were present. Comparatively few specimens of the parasite appear to be capable of producing quite marked symptoms of disease in onion seedlings.

Field beans. Symptoms of attack were similar to those noted above by direct invasion from oats though in this case they were not so severe. There was distortion of one or more of the leaf blades with small scattered brown lesions marking the site of the original entry of the parasite into the tissues. A few adult *A. dipsaci* were found in the vicinity of such lesions. The base of the stem and the lowest stipules showed marked brown lesions and in one of the stipules adults of the parasite were plentiful together with numerous free eggs.

Mangolds. Several seedlings showed similar symptoms to those in the oat inoculum row. One of these, stained in Scarlet R. had extensive brown lesions in the cotyledons, the cotyledon petioles and in the stem surrounding the primary shoot which was more or less destroyed. Many adult examples of *A. dipsaci* and some free eggs were present in the cotyledons themselves.

Red clover. Several seedlings manifested more or less severe damage to the stem, the cotyledon petioles, the cotyledons themselves and around the terminal shoot. Groups of mainly pre-adult and some adult *A. dipsaci* were found on dissection but no gravid females or free eggs were present.

BLACK BINDWEED INOCULUM.

Oats. Two or three seedlings showed mild symptoms of "tulip-root" with some stunting and swelling of the leaf-base region. One

seedling stained in Scarlet R. had several adult *A. dipsaci* and free eggs in the inflated tissues of the leaf base of one of the outermost leaves, thus proving that there was a light but definite infection.

Onions. A few seedlings showed mild symptoms of "bloat". One of these after staining in Scarlet R. showed a few adult *A. dipsaci* and free eggs in the inflated tissues.

Field beans. The plants showed similar symptoms to those in the cleavers row but they were less marked. One seedling had small brown lesions on the lower stipules in the vicinity of which a group of adult *A. dipsaci* and some free eggs were found; thus proving a light but definite infection.

Mangolds. Similar symptoms to those found in the oats and cleavers inoculations but the attacks rather lighter. One or two seedlings dissected had *A. dipsaci* present in lesions of the stem, the petioles of the cotyledons and in the tissues around the terminal shoot. Adults of the parasite and some free eggs were found in the cotyledons themselves.

Red clover. Many seedlings showed damage similar to that already noted in the case of the oat and cleavers inoculations. Two or three seedlings dissected after staining with Scarlet R. had severe brown lesions on the inner side of the cotyledon petioles and in the tissues around the terminal bud which, again, was more or less deformed. In the lesions a few pre-adults of *A. dipsaci* were found but no gravid females and no free eggs.

2. With the infected specimens of common chickweed and cleavers collected on the same farm on the occasion of the second visit, a further cross-infection experiment was set up. The amount of infected material to serve as inocula was rather limited so two 3-in. pots only were used, with a third, without inoculum, to serve as control. The procedure followed was the same as before; the dried, infected fragments of cleavers and common chickweed being distributed as evenly as possible on the surface of the soil in the pots. This was lightly covered with soil and on this onion seed, var. Best of All, was sown and covered with a further $\frac{1}{2}$in. of soil. The pots were finally watered from below and plunged in a bed of ashes out of doors. Seedlings developed evenly in all three pots but by the 16th day after sowing some of them in the two inoculated pots were showing obvious signs of "bloat" and it was clear that the parasite could readily transfer itself from these two weeds to onions with accompanying disease symptoms. The photograph reproduced

at the end of this paper was taken 20 days after the seed had been sown and shows healthy seedlings in the middle with two mildly bloated seedlings from the common chickweed pot on the left and two rather more seriously affected seedlings from the cleavers pot on the right. It should not be inferred from this picture that disease was less severe in the chickweed pot than the cleavers pot as on the 16th day after sowing two seedlings in the chickweed inoculated pot were as stunted and swollen as those shown from the cleavers. By the 20th day they were too badly affected to make a good photograph with the result that two less seriously diseased seedlings were included in the picture.

3. In the two cross-infection experiments already described the parasite came from an oat source; either from oat itself or from weeds occurring on land infested with what has been spoken of as the oat race of the parasite. In the third experiment, now to be described, the infective material came from a diseased onion source. The inoculum consisted of diseased specimens of scarlet pimpernel (*Anagallis arvensis* L.) collected from the onion "bloat" plot at this Institute, as described above p. 4. These stunted and much deformed plants were brought into the laboratory on 27.vii.'44 and slowly dried at room temperature. On 27.ix.'44 they were broken up into small pieces and used as an inoculum by distributing them on the surface of the soil in 4 5-in. flower pots which had been filled with soil to within about 1in. of the rim. A thin layer of soil was placed on the top of this material and seeds of the following were then sown, one pot to each host plant tested: onion (var. Rousham Park Hero); oats (var. S.147); field bean (var.?); English red clover. The seeds were covered with soil, the pots watered from below and finally plunged up to the rim in a bed of ashes out of doors. For the first fortnight after sowing they remained in the open but were afterwards brought into a cool greenhouse. On 8.xi.'44 *i.e.* after 41 days, they were examined with the following results.

Oats. Of three seedlings growing one was seriously diseased, being stunted, swollen and discoloured at the base. On carefully teasing the tissues of the leaf-base apart, under the binocular microscope, large numbers of adult *A. dipsaci* with many free eggs were found. This was a clear case of transference of the parasite, of onion/scarlet pimpernel origin, to oats with the production of typical disease symptoms.

Onions. Two typically bloated and distorted seedlings were dissected and were found to contain adult specimens of *A. dipsaci*.

Field beans. Four plants growing from the 4 seeds sown each showed yellowish-brown lesions on one or more leaf blades with some twisting of the leaf lamina. One plant was removed by severing the stem just below soil level. The two stipules, at this level, on either side of the stem, showed brown lesions in the vicinity of which good numbers of adult *A. dipsaci* were found as well as many eggs and larvae. In a similar lesion on the stem and from one on a lower leaf blade further adults, eggs and larvae were obtained and it was clear that there had been a successful transfer of the parasite to field bean.

Red clover. Several seedlings were obviously stunted whilst others were practically normal in appearance. Five affected ones were lifted and examined in water under the binocular microscope. In each case there were more or less marked brown lesions on the cotyledon petioles and on the main stem around the primary central shoot. One or two of the seedlings showed stunting of the petioles of the first true leaves. Examples of *A. dipsaci* were found in the vicinity of all these lesions but not within the tissues of the cotyledons themselves or the first true leaves. None of the females was in a gravid condition and no eggs were present.

Discussion.

Various field and plot observations set out earlier in this paper show that the stem eelworm attacking oats readily transfers to field beans and also to onions and back from onions to oats. It can transfer from oats to rhubarb and vice versa and it has been shown to be capable of transference, under experimental conditions, from rhubarb to onion seedlings with the production of "bloat" symptoms. Parsnips also may become seriously diseased when grown after bloated onions. The following weeds have been found either by Johnson, Staniland or the writer, when occurring amongst eelworm infested oats, field beans and onions, to be capable of serving as reservoir hosts in which an infestation of the parasite can be maintained from year to year in the absence of a susceptible crop host, namely, common chickweed (*Stellaria media* Cyrill.); cleavers (*Galium Aparine* L.); black bindweed (*Polygonum Convolvulus* L.); scarlet pimpernel (*Anagallis arvensis* L.); mouse-ear chickweed (*Cerastium vulgatum* L. sens. lat.); field mouse-ear chickweed (*Cerastium arvense* L.); and thyme-leaved sandwort (*Arenaria serpyllifolia* L.). Infested specimens of cleavers and black bindweed

have been used by the writer as inocula in cross-infection experiments with the result that the parasite, of oat/weed origin, has been successfully transferred from these two weeds to seedlings of oats, onions and field beans with the production of characteristic disease symptoms. From this source too it will invade and reproduce successfully in seedlings of mangold but fails to reproduce in red clover seedlings though it may severely damage them. From infested common chickweed and cleavers, also of oat/weed origin, infestation was successfully set up in onion seedlings with the production of "bloat" symptoms. By using badly infested plants of scarlet pimpernel from an onion plot the parasite was readily transferred to oats, field beans and onions. Invasion of red clover seedlings took place in this experiment with damage to the tissues but the eelworm did not reproduce in them. All the results of the foregoing observations and experiments clearly indicate that this race of the stem eelworm is interchangeable between oats, field beans, onions, parsnips and rhubarb. To these may probably be added mangolds since the parasite successfully reproduced in tissues of mangold seedlings. Red clover, however, is not successfully parasitized by this race. Proof has been obtained by pot experiments that from such infested weeds as common chickweed, cleavers, black bindweed and scarlet pimpernel the parasite can transfer to seedlings of oats, field beans, onions and mangolds and the rôle of these weeds as reservoir hosts is thus fully confirmed.

References

Bos, J. Ritzema, 1892.—"L'Anguillule de la Tige (*Tylenchus devastatrix* Kühn) et les Maladies des Plantes dues à ce Nematode. Annotations : Maladie vermiculaire des fèves (*Vicia faba*)." *Arch. Mus. Teyler.* Ser. II, Vol. 3., pp. 563–569. (W. L. 1874).

Goodey, T., 1929.—"The stem eelworm *Tylenchus dipsaci* (Kühn, 1858) ; Observations on its attacks on potatoes and mangolds with a host-list of plants parasitized by it." *J. Helminth.* VII (4), 183–200. (W. L. 11224b).

1937.—"On some new hosts of the stem eelworm *Anguillulina dipsaci*." Ibid, XV (4), 215–220.

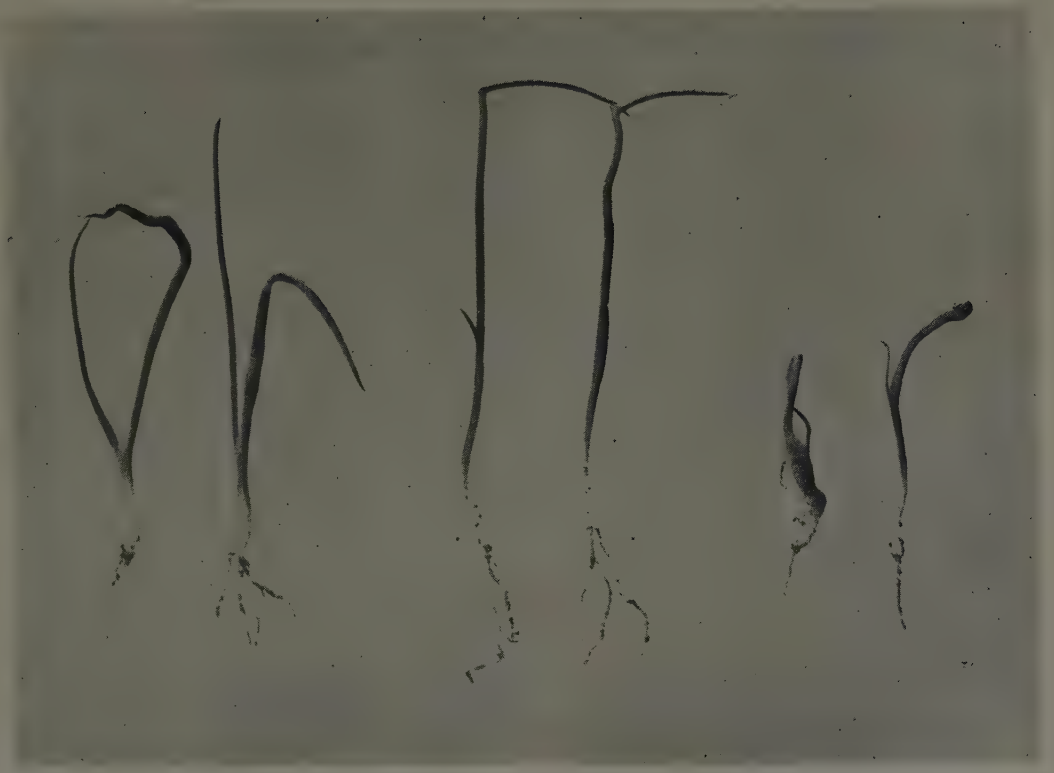
1941.—"*Anguillulina dipsaci* from 'tulip-root' oats injuring seedlings of a seeds mixture." Ibid, XIX (1/2), 1–8. (W. L. 11224b).

Johnson, L. R., 1936.—"Observations on the occurrence of *Anguillulina dipsaci* (Kühn, 1858) on rhubarb in Yorkshire." Ibid, XIV (2), 77–84.

1936a.—"A note on the occurrence of *Anguillulina dipsaci* (Kühn, 1858) on certain weeds, including a new host record." Ibid, XIV (4), 233–235.

1940.—"On the stem and bulb eelworm (*Anguillulina dipsaci* Kühn) with special reference to its occurrence on weeds of arable land." *Ann. appl. Biol.*, 27 (2), 248–251. (W. L. 1025).

Staniland, L. N., 1945.—"The occurrence of *Anguillulina dipsaci* (Kühn) on weed hosts, including new hosts records in fields of oats affected by 'tulip-root.'" Ibid. 32 (2), 171–173.



'Bloat' of onion seedlings, 20 days after sowing, caused by *Anguillulina dipsaci*, from weed hosts; chickweed on left, cleavers on right, healthy seedlings in centre.

Journal of Helminthology, Vol. XXII, No. 1, 1947, *pp.* 13—28.

Progress of the *Diphyllobothrium* Epizootic at Poulaphouca Reservoir, Co. Wicklow, Ireland.

By M. D. Hickey, M.D., M.Sc., M.R.C.P.I., D.P.H.

(*Department of Pathology, Mater Misericordiae Hospital, Dublin and the Departments of Pathology and Bacteriology, University College, Dublin*)

and

J. R. Harris, F.R.E.S.

(*Department of Zoology, Trinity College, Dublin*).

Introduction.

The occurrence of two species of the cestode *Diphyllobothrium* at Poulaphouca Reservoir, Co. Wicklow, Ireland, has already been recorded (Harris & Hickey 1945). This paper contains the results of an investigation of the epizootic.

The reservoir was formed by the erection of a dam on the River Liffey which resulted in the inundation of approximately 8 square miles of land. Backing commenced in March 1940 and the water had risen to within a few feet of its maximum height by June 1942. From the rivers and streams submerged the reservoir became stocked with trout (*Salmo trutta*), minnow (*Phoxinus phoxinus*), sticklebacks (*Gasterosteus aculeatus*) and loach (*Nemachilus barbatula*).

During 1943 many dead trout were observed in the reservoir. In December 1943 (Hickey & Harris, 1944) it was found that these trout contained plerocercoids of the Diphyllobothriidae. A similar mortality of trout occurred in South Wales (Duguid & Sheppard, 1944). The report from South Wales contained an account of the parasite. It has since been suggested (Baylis, 1945) that the species involved there was *Diphyllobothrium dendriticum* (Nitzsch, 1824).

In the Poulaphouca outbreak two species of *Diphyllobothrium* were found. Baylis (1945) has pointed out that the identification of these worms is uncertain. He has tentatively identified them as *D. dendriticum* (Nitzsch, 1824) and *D. ditremum* (Creplin, 1825). In this paper these tentative determinations will be followed but it is apparent that they may have to be amended at some future date when the taxonomy of this genus has been worked out. A collection, comprising most of the adult cestodes obtained from each of the naturally

occurring definitive hosts has been presented to the British Museum (Natural History).

During the course of these investigations the authors were fortunate in obtaining adult specimens of *D. latum* from man, and plerocercoids of this species from fish, in the Co. Leitrim area (Harris & Hickey, 1945). This additional material was valuable for comparative purposes.

Progress of the Outbreak.

Dead trout were first observed in May 1943 and soon became very numerous. During the following winter the number of dead fish seen diminished greatly. In the spring of 1944 mortality again became noticeable and the epizootic reached its maximum from May to September of that year. Deaths practically ceased towards the end of September but were again apparent, though in much smaller numbers, in the summer of 1945. They again stopped in the winter and to date of writing (December 1946) no further mortality has become apparent. The lake is still well stocked with trout.

Infection in the Fish.

During 1944, 108 trout found dead were examined. Of these fish 103 ranged from 30 to 50cms. in length. These were all infected with plerocercoids, each fish contained usually from 100 to 300 parasites. The remaining 5 trout were under 30cms. in length, furunculosis was present in two, and in three the cause of death was not found. Plerocercoids were absent from four of these trout and the fifth contained a single parasite.

The majority of the infected trout showed a plastic peritonitis. Blood stained ascitic fluid was present in approximately 25%. Oedematous granulation tissue, frequently containing large extravasations of blood, was present on the stomach or intestine in about 80% of cases, often in sufficient amount to bind the stomach and pyloric caeca into a large tumour-like mass. Adhesions between the abdominal viscera and parietes were a frequent finding. Marked pallor of the flesh and gill rays was almost constant suggesting severe anaemia.

In addition to these dead and dying fish, 146 trout were caught alive by rod and line or by net. Of these fish 18 were less than 25cms. in length and were not infected, 28 were over 40cms. in length and were all infected. The remaining 100 fish showed increasing infection in direct relation to their length. (See Table I and Graph.)

In these trout plerocercoids were usually located in the subserous tissue of the abdominal viscera, rarely on the surface of the skeletal muscles external to the parietal peritoneum and only once actually in the skeletal muscles. In the last case two plerocercoids were found in

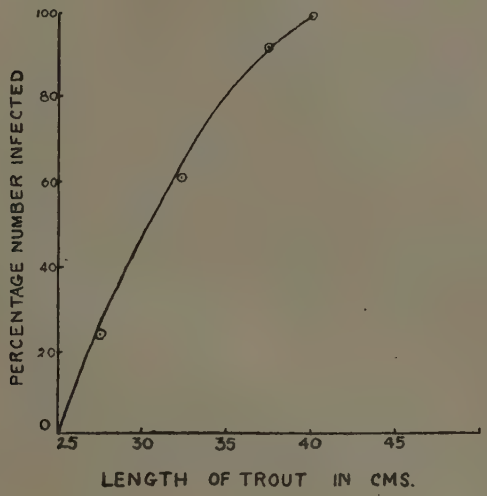


TABLE I.

Length of fish in cms.	Under 25 cms.	25–30	30–35	35–40	40 and over
Number examined ..	18	22	52	26	28
Number infected ..	0	5	32	24	28
Percentage infected ..	0	23	62	92	100

the body muscle near the peripheral end of a thick peritoneal adhesion.

In infected fish the worms are usually visible under the peritoneum and appear as small white nodules. These nodules are most common on the stomach wall or in the subperitoneal connective tissue in the region of the pyloric caeca. On section the plerocercoid is sometimes surrounded by a dense layer of fibrous tissue. In other cases this layer is absent.

When granulation tissue was extensive the plerocercoids contained in it were often found extended instead of being coiled up or encysted. On section, such tissue usually showed many vacant cysts or burrows which had been formed by plerocercoids migrating elsewhere.

Sticklebacks in the reservoir also carried the infection, 75 out of 545 (13.8%) examined were infected. Usually only one parasite was present, either encysted in the viscera or else lying loose in the body cavity. This latter condition was more usual when the fish were not examined for many hours after death. Multiple infection was present in a few cases, but the greatest number of plerocercoids found in any one stickleback was five. *Schistocephalus solidus* larvae were also frequently encountered in the sticklebacks, this parasite was found in 72 out of a total of 349 sticklebacks (20.6%).

Minnow and loach, numbering 143 and 56 respectively, were also examined, but none was found infected. This accords with Janicki & Rosen's (1917) finding relative to *D. latum* that Cyprinids do not act as host species.

The Plerocercoids.

The plerocercoids superficially are similar to those described by Duguid & Sheppard (1944). Both the non-segmented and the segmented types described by those authors were present. The longest plerocercoid encountered measured 34cms. when relaxed and showed distinct segmentation. The smallest segmented plerocercoid found in fish was 2.7cms. long and the majority exceeded 4cms. Non-segmented plerocercoids ranged from 0.2cms. to 9.0cms. in length.

The Definitive Hosts.

Naturally infected definitive hosts of *D. dendriticum* and *D. ditremum* occurring in the area have been recorded (Hickey & Harris, 1944). Subsequently the heron was found to be a host of *D. ditremum*. Details are given in Table II.

Amongst these were 45 Black-headed Gulls, three Little Grebe and two Tufted Duck. It was noted that the cestode *Schistocephalus solidus* was frequently encountered in the Black-headed Gulls, as many as 33 specimens being found in a single bird. More than 50 specimens were present in one of the Little Grebe and the Tufted Duck also harboured them.

Black-headed Gulls were frequently observed feeding on dead trout. On a few occasions sticklebacks were found in the gastric contents of these birds.

TABLE II.

Species of Bird	No. Examined	No. Infected	Species of parasite present
Greater Black-backed Gull *Larus marinus*	22	15	*D. dendriticum*
Lesser Black-backed Gull *Larus fuscus*	20	11	„ „
Herring Gull *Larus argentatus*	12	4	„ „
Cormorant *Phalacrocorax carbo*	13	11	*D. ditremum*
Shag *Phalocrocorax graculus*	3	2	„ „
Heron *Ardea cinerea*	13	2	„ „

Specimens of the following other possible avian hosts were examined with negative results.

Kingfisher ... *Alcedo ispida*
Dipper ... *Cinculus aquaticus*
Little Grebe ... *Podicipes fluviatilis*
Wild Duck ... *Anas boscas*
Teal ... *Querquedula crecca*
Wigeon ... *Mareca penelope*
Shoveler ... *Spatula clypeata*
Black-headed Gull ... *Larus ridibundus*
Tufted Duck... *Fuligula cristala*
Swan ... *Cygnus musicus*
Water Hen ... *Gallinula chloropus*
Raven ... *Corvus coras*
Hooded Crow *Corvus cornix*
Rook ... *Corvus frugilegus*
Jackdaw ... *Corvus monedula*
Tern ... *Sterna fluviatilis*

Considerable variation was found in the numbers of *Diphyllobothrium* present in the definitive hosts. Of the 45 infected birds 26

contained mature adult parasites,* the remainder contained only plerocercoids or immature adults. The greatest number of mature adult specimens of *D. dendriticum* found in a single bird was 11, and of immature adults 51. Similar figures for *D. ditremum* were 40 and about 1,000 respectively.

A total of 60 mature adult parasites and over 111 immature adults were examined from gulls, and 94 mature adults and over 2,500 immature adults from cormorants and shags.

All the adults, both mature and immature, from the former birds were recognizable as *D. dendriticum* and from the latter as *D. ditremum.*

It was noted when examining these avian hosts that segmented plerocercoids up to 6cms. in length were very frequently encountered in gulls, and that slightly smaller unsegmented specimens were also often present. All plerocercoids located in cormorants and shags, however, were less than 2.5cms. long and parasites longer than this were invariably immature adult specimens.

Natural Infection of Definitive Hosts.

The feeding habits of the avian hosts were considered in relation to the manner in which they became parasitized. During field work a close watch was kept on the birds concerned, and the following observations were recorded.

1. Cormorants and shags fed only on living trout, which they captured in the reservoir by diving and pursuing the fish under water. All of these birds examined contained trout remains in their stomachs and no other fish were encountered (with the exception of one small conger eel found in the stomach of a cormorant which had apparently only just arrived at the reservoir). Dead trout were often left around the shore of the reservoir and at certain known places where the cormorants regularly rested, but such fish were invariably ignored by these birds.

2. Black-headed and Herring Gulls on the other hand, fed regularly on dead trout and were never observed to attack any fish unless it was

* Adult parasites were considered mature when the reproductive organs had fully developed in at least some of the proglottids and the uteri contained eggs. Immature adults were those in which the reproductive organs had not fully developed but in which the developing proglottids had assumed the shape of mature proglottids. Adults with such proglottids could be recognised at a glance as the posterior edge of each proglottid was invariably wider than the anterior.

TABLE III

Species	No. of specimens measured	Length of Scolex in m.m.		Width of Scolex expressed as % of length		Depth of Scolex expressed as % of length	
		Mean and S.D.	Range	Mean and S.D.	Range	Mean and S.D.	Range
D. dendriticum	28	1·59 ± 0·27	1·15—2·30	24 ± 4·4	16—34	42 ± 7·1	28—56
D. ditremum	47	1·72 ± 0·18	1·32—2·05	50 ± 6·9	36—67	69 ± 8·8	50—90

In relation to the strobila the bothria of the scolex open ventrally and dorsally and in the table the dorso-ventral diameter of the scolex is referred to as the depth.

already dead or else in a dying condition. These gulls sometimes swallowed small trout whole, but more usually they only ate the gills and viscera of the fish.

3. Trout remains were found in herons only during the winter months, and it is probable that such trout were captured while spawning on the shallow reaches of the rivers.

The Adult Parasites.

The taxonomy of the adult parasites is not considered here in detail, but certain general distinguishing features between *D. dendriticum*, *D. ditremum* and *D. latum* are noted. For comparative purposes the cestodes were killed and relaxed in tap water and then fixed in 10% formol saline.

The scolices of *D. dendriticum* and *D. latum* superficially resemble each other closely, but that of *D. ditremum* shows a marked difference. In lateral view the two former are almond shaped whereas the latter is ovoid or more nearly circular. (See Figs. 2 and 4.) This difference is sufficiently apparent to allow it to be easily perceived by the naked eye, and factual evidence is contained in the table of measurements (Table III). Additional distinguishing features are found in the formation of the neck of each species. *D. dendriticum* and *D. latum* each possess a relatively long neck (usually longer than the scolex, 0.5 to 10.0mms.) and the exact location at which segmentation becomes evident is extremely difficult to define. The neck of *D. ditremum* is relatively very short (much shorter than the scolex, 0.2 to 0.4 mms.) and it is clearly demarcated from the anterior proglottids of the strobila.

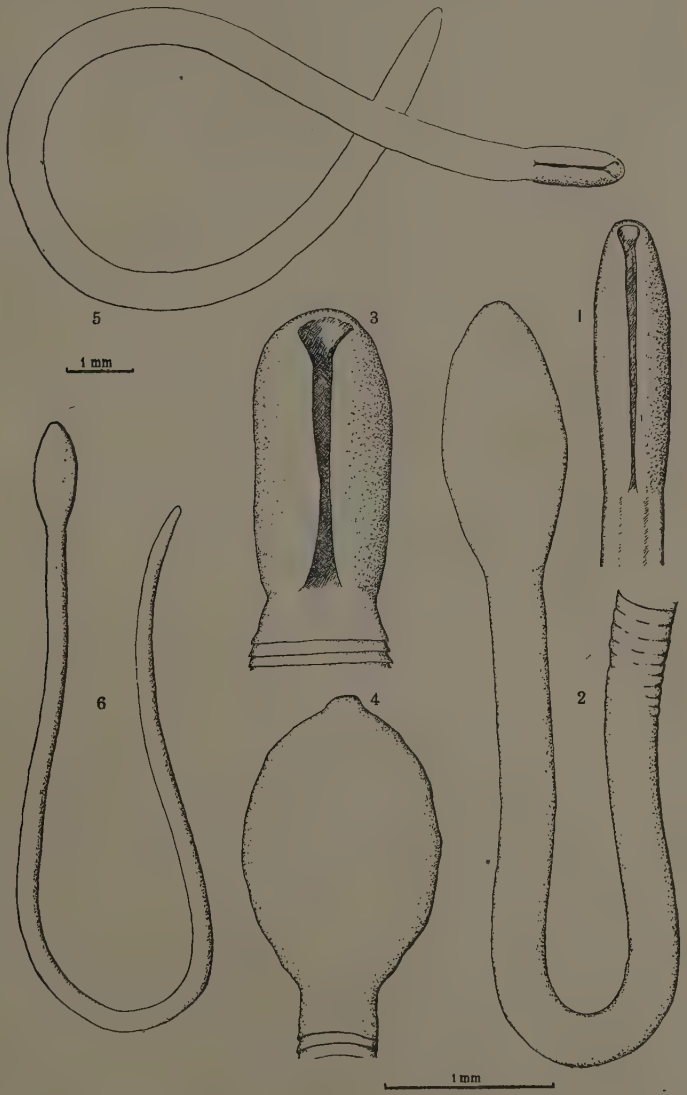
Both *D. dendriticum* and *D. ditremum* differ from *D. latum* in the arrangement of the testes. In the latter the testes are arranged in two lateral fields which remain separate from each other along the whole strobila. In the former two species the lateral fields become confluent anterior to the cirrus sac in each proglottid.

Little importance is attached to variations in size between mature adult specimens of *D. dendriticum* and *D. ditremum* as this would be

Figs. 1–4 show scolex, neck and anterior proglottids.

Adult *D. dendriticum*
1. Dorso-ventral view. 2. Lateral view.
Adult *D. ditremum*
3. Dorso-ventral view. 4. Lateral view.
Plerocercoid from trout.
5. Dorso-ventral view. 6. Lateral view.

The larger scale refers to Figs. 1 to 4.


5
1 mm
3
1
6
4
2
1 mm

affected by the number of parasites carried by the respective definitive hosts. The lengths of the six largest specimens of *D. dendriticum* varied from 51 to 75cms. The number of proglottids in each of 14 mature individuals varied from 214 to 447 with an average of 318. Similar figures for *D. ditremum* were lengths 15 to 30.5cms. and number of proglottids in 18 mature individuals 75 to 146 with an average of 118.

Feeding Experiments.

Chicks of Herring Gulls, a Greater Black-backed Gull and a Shag were obtained from the sea coast. The birds were kept for three weeks and their faeces then examined and found free of *Diphyllobothrium* eggs. Three of the Herring Gulls were given respectively 1, 1 and 3 feeds of plerocercoids with negative results. The remaining birds were given larger numbers of plerocercoids. Details and results are shown in Table IV.

Feeding experiments were also carried out on six albino rats, six adult dogs, one cat and six kittens.

Rats. Two rats were given one feed each, four rats were given five feeds each over a period of five days. The faeces were examined for ova at weekly intervals and the animals were killed from 26 to 30 days after the last feed. All results were negative.

Dogs. The six dogs were fed as follows. No. 1 two feeds over a period of five days. No. 2 four feeds in four days. No. 3 three feeds in eight days. No. 4 seven feeds in eleven days. Nos. 5 and 6 six feeds each in six days. Dog No. 1 received four plerocercoids and dog No. 2 twelve. The remaining four dogs received between 200 and 300 plerocercoids each. All results were negative.

Two further dogs fed with *D. latum* plerocercoids served as a control. As already reported (Harris & Hickey, 1945) these latter were successfully parasitized.

Cat and Kittens. The cat was given four feeds. Result negative. A six months old kitten was given six feeds. Result negative.

Five two months old kittens were given from one to three feeds each. Two of these gave negative results. One passed *Diphyllobothrium* ova 18 days after the first feed but was negative at autopsy 89 days after feeding. The remaining two each contained two adult specimens of *D. dendriticum* at autopsy 27 and 38 days after the first feed. These last two kittens had each received three feeds.

TABLE IV.

Species of Bird	Details of feeds given	Results. Time given from first day of feeding
Herring Gull	3 feeds over a period of 12 days.	*Diphyllobothrium* ova in faeces from 44th to 105th day. Killed 136th day no parasites found.
„ „	4 feeds over a period of 12 days.	Faeces positive from 46th to 105th day. Killed 137th day no parasites found.
„ „	4 feeds over a period of 12 days.	Faeces positive 36th day. Killed 99th day 4 specimens *D. dendriticum* present.
„ „	6 feeds over a period of 10 days.	Negative.
„ „	6 feeds over a period of 10 days.	Faeces positive 47th day.
Greater Black-backed Gull	4 feeds over a period of 12 days.	Killed 45th day 4 specimens *D. dendriticum* present.
Shag	5 feeds over a period of 16 days.	Killed 19th day 4 adult specimens *D. ditremum* 6 immature specimens and 13 plerocercoids present.

THE EGGS AND CORACIDIA.

The eggs of both *D. dendriticum* and *D. ditremum* are ovate, amber coloured and operculated. When placed in 10 to 20mms. of tap water, kept in a good light and at room temperature during the summer they hatch in from 9 to 15 days. Eggs kept under similar condition but in winter in an unheated room may take as long as two months. It was found that eggs will ripen but not hatch when kept in the dark, but when such ripe eggs are placed in sunlight or artificial light they hatch within a few minutes.

The coracidium of *D. dendriticum* has been described by Duguid & Sheppard (1944). No differences were observed in the structure of any of the three species studied except that *D. latum* coracidia were slightly larger.

First Intermediate Host.

Cyclops strenuus abyssorum and *Diaptomus gracilis* were each infected with all three species of coracidia. Experiments were carried out with other copepods and it was found that some species such as *C. viridis* could not be induced to eat the coracidia and other species, *e.g.*, *C. albidus* fed heavily on the coracidia but did not become infected. On dissection, the gut of *C. albidus* was found to contain as many as 20 disintegrating coracidia or onchospheres.

The onchospheres of all three species developed to the procercoid stage in the copepod *D. gracilis* but infection of fish from these copepods has not yet been effected. One procercoid which appeared to be a *Diphyllobothrium* species was found in a specimen of *D. gracilis* taken from the reservoir.

Discussion.

During the period of the investigation a noticeable feature was the seasonal fluctuation in mortality among the trout. Mortality was due to peritonitis caused by the plerocercoids leaving their burrows or cysts and migrating through the surrounding tissue. It was suspected that temperature influenced the activity of the plerocercoids and this was demonstrated by the following experiments.

(1) Plerocercoids which were placed in physiological saline were found to be non motile at 10° C., sluggishly motile at 12° C. and active at 14° C.

(2) The viscera of a heavily infected trout were kept for several days in saline at 5° C. During this period the plerocercoids remained within their cysts and showed no evidence of activity. The viscera were then placed in saline at a temperature of 15° C. and many of the plerocercoids immediately became active and within a few minutes had left their cysts.

It is thus easy to understand why the mortality was confined to the warmer months.

The freedom from infection of trout under 25cms. in length and the correlation between trout of various lengths greater than this and the severity of their infection is of interest. The explanation probably lies in the feeding habits of the fish.

Autopsies of fish from the reservoir, Harris (unpublished) show that :

(1) Sticklebacks commonly fed on Copepoda.

(2) Evidence was not obtained that any trout ever fed on these small crustaceans (the smallest trout examined was 12cms.).
(3) Trout over 25cms. in length fed extensively on sticklebacks but trout under this length seldom did so.
(4) The number of sticklebacks consumed by trout increased in proportion to the size of the trout. As many as 16 sticklebacks were found in one trout of 52cms.

Brumpt (1936) records that the amphibian hosts of *D. mansoni* and the fish hosts of *D. latum* can become infected through ingesting young plerocercoids but that such refixation of the intermediate hosts does not occur with "adult" plerocercoids. Unsworth (1944) has demonstrated that fish hosts become infected through ingesting plerocercoids of *D. dendriticum* parasitizing a smaller fish, and that sticklebacks become infected with plerocercoids of *D. dendriticum* through eating infected Copepoda. As 13.8% of the sticklebacks examined from Poulaphouca Reservoir carried the parasite it is probable that the trout became infected as a result of feeding on these small fish, and that the severity of their infection was directly proportional to the number of sticklebacks consumed, *i.e.* to the size of the trout.

Scale readings of trout caught in the reservoir, Harris (unpublished) show that most of the heavily infected, *i.e.* the larger fish, had not entered the reservoir until they were two, three or four years old, and it might be considered that such fish had carried the infection into the reservoir with them, as it is known that trout *fry* at times feed on Copepoda. It is unlikely, however, that this occurred, as the former habitat of such fish must have been either the Upper Liffey or one of its tributaries. In a fluviatile environment of the type of these rapid mountain streams Copepoda are relatively scarce and belong to the benthic fauna, whilst the coracidia are essentially planktonic and therefore it is unlikely that Copepoda would become parasitized.

How the infection was introduced into the reservoir has not been determined. Subsequent to the formation of the reservoir cormorants and gulls arrived there in considerable numbers. As these birds are known to frequent another reservoir in which the trout were carrying the disease, Harris & Hickey (1945) and which is situated only about 15 miles from Poulaphouca Reservoir it is probable that the infection was introduced by the avian hosts.

Luhe (1910), Joyeux and Baer (1936) record that *L. argentatus* is one of the host species for both *D. dendriticum* and *D. ditremum.*

The findings in this paper definitely show that *L. argentatus* is a host species of *D. dendriticum* but not of *D. ditremum*. It would appear, therefore, either that the earlier record of the determination of this definitive host of *D. ditremum* is incorrect, or else that the *Diphyllobothrium* occurring in Poulaphouca is not *D. ditremum*.

A further anomaly lies in the records (Luhe 1910) and (Joyeux & Baer, 1936) that *L. ridibundus* is a host species of *D. dendriticum*. The findings at Poulaphouca do not agree with this. It has been recorded in this paper that 45 specimens of *L. ridibundus* from Poulaphouca were examined but that none of them was found to harbour species of *Diphyllobothrium*. It is also shown that these birds were commonly infected with *Schistocephalus solidus*—as many as 33 of the cestodes being located in one bird. As sticklebacks were the only fish in the reservoir which harboured *Schistocephalus solidus* it follows that the Black-headed Gulls must have been feeding on sticklebacks and must also have ingested many *Diphyllobothrium* plerocercoids. It would, therefore, seem probable that these birds do not act as hosts of either of the two species of *Diphyllobothrium* occurring in Poulaphouca.

One probable distinguishing feature between the two species of plerocercoids was noticed. It has been stated that segmented plerocercoids up to 6cms. in length were very frequently encountered in gulls and that slightly smaller unsegmented specimens were also often present. Also that all plerocercoids located in cormorants and shags were less than 2.5cms. long. When it is considered that the smallest segmented plerocercoid found in fish was 2.7cms. long and that the majority exceeded 4cms., it would appear that it is only specimens of *D. dendriticum* which develop segmentation while in the plerocercoid stage in fish and also that plerocercoids of *D. ditremum* in fish do not exceed 2.5cms. in length.

It is of interest that *D. ditremum* was found only in those birds which pursued and captured trout which were alive and not necessarily incapacitated by parasites, whilst *D. dendriticum* was restricted to birds which fed mainly on dead or dying trout.

This suggests that it is only the plerocercoids of *D. dendriticum* which have a fatal effect on the trout, and that unless they brought about mortality in the fish they would be unable to complete their life cycle.

Unsworth (1945) records that he successfully infected two puppies with plerocercoids obtained from fish from Poulaphouca Reservoir

and comments on the fact that the cestodes are capable of reaching maturity in both a bird and a mammal.

It has been recorded in this paper that the results of feeding experiments on six rats, six adult dogs and one cat were negative, but that positive results were obtained with three out of six kittens. These findings are in accordance with the well known fact that the young of mammals are more easily parasitized than older animals of the same species. Malformations in the parasites obtained from these mammalian hosts suggest that such hosts are abnormal (Baylis, 1945).

Summary.

1. The pathology of the infection in the trout of Poulaphouca Reservoir is described.

2. An annual mortality was found to occur in the trout during the summer months. This was caused by peritonitis which is attributed to plerocercoids becoming active as a result of the increase in temperature.

3. It is demonstrated that the infection in trout is probably of a secondary nature resulting from their feeding on sticklebacks primarily infected from the Copepod hosts.

4. The tentative determination of Baylis (1945) that the two species of *Diphyllobothrium* occurring in Poulaphouca are *D. dendriticum* and *D. ditremum* is followed.

5. It is shown that of 22 possible species of avian hosts examined only *Larus marinus, L. fuscus* and *L. argentatus* harboured *D. dendriticum* and only *Phalacrocorax carbo, P. graculus* and *Ardea cinerea* harboured *D. ditremum.*

6. The records of earlier authors that *L. argentatus* is a host of *D. ditremum* and that *L. ridibundus* is a host of *D. dendriticum* are queried, and it is suggested either that the former of these records is incorrect, or else that the cestode from Poulaphouca which has been tentatively identified as *D. ditremum* is some other species.

7. Morphological and ecological differences among the plerocercoids suggest a means of distinguishing between the two species present in the reservoir. It is suggested that only plerocercoids of *D. dendriticum* cause mortality in trout.

8. Some differentiating taxonomic features of the adult cestodes *D. dendriticum, D. ditremum* and *D. latum* are described.

9. Feeding experiments on birds and mammals are recorded and confirm the determination of the definitive hosts species ascertained in field work.

10. Infection of *Cyclops strenuus abyssorum* and *Diaptomus gracilis* was effected with coracidia of all three species of *Diphyllobothrium* obtained.

Acknowledgements.

The writers are indebted to Professors J. Brontë Gatenby and W. D. O'Kelly for their advice and interest during the course of the investigation.

They also desire to thank Professor T. G. Browne and Mr. F. St. G. Sleith of the Veterinary College of Ireland for their helpful co-operation and facilities for keeping animals; Dr. H. A. Baylis of the British Museum for examining specimens of the parasites and for his general interest in the work; the numerous anglers and other interested people who provided facilities, collected material and helped in many other ways. In this connection the writers are particularly indebted to Professor T. T. O'Farrell and Dr. W. R. O'Farrell; Messrs. W. F. Richardson and D. Preston and to Dr. J. M. Clearkin of Blessington.

One of us (M.D.H.) was in receipt of a grant from the Medical Research Council of Ireland.

References

Baylis, H. A., 1945.—" On the probable identity of a Cestode of the genus *Diphyllobothrium* occurring in Wales and Eire." *Ann. trop. Med. Parasit.* 39, 41–45. (W.L. 1063.)

Brumpt, E., 1936.—" Précis de Parasitologie," Paris. pp. 800–813.

Duguid, J. B. and Sheppard, E. M., 1944.—" A *Diphyllobothrium* Epidemic in Trout." *J. Path. Bact.* 56, 73–80. (W.L. 11431.)

Harris, J. R.—Unpublished data.

Harris, J. R. and Hickey, M. D., 1945.—" Occurrence of the Diphyllobothriidae in Ireland." *Nature, Lond.* 156, 477–478. (W.L. 14900.)

Hickey, M. D. and Harris, J. R., 1944.—" Definitive Hosts of a Species of *Diphyllobothrium* causing mass infection of Trout in Reservoirs." Preliminary Note." *Brit. med. J.* 2, 310. (W.L. 3579.)

Janicki, C. and Rosen, F., 1917.—" Le Cycle evolutif du *Bothriocephalus latus* L." *Bull. Soc. neuchâtel, Sci. nat.*, 42, 19–53. (W.L. 5299.)

Joyeux, C., and Baer, J. G., 1936.—" Faune de France." Cestodes 30, 335–336.

Luhe, M., 1910.—" Parasitische Plattwurmes II Cestodes " in Brauer's *Die Susswasserfauna Deutschlands* Heft. 18, 142, 20–21.

Unsworth, K., 1944.—" Observations on the life cycle of a Species of *Diphyllobothrium* found parasitizing Trout in Great Britain." *Ann. trop. Med. Parasit.*, 38, 213–219. (W.L. 1063.)

Journal of Helminthology, Vol. XXII, No. 1, 1947, *pp.* 29—36.

On *Viannella africana* n.sp., *Cheilospirura falconis* n.sp., and *Oesophagostomum tridentatum* Maplestone, 1932.

By Phyllis A. Clapham, D.Sc.

(*From the Institute of Agricultural Parasitology, St. Albans*).

The nematodes described below are in the collection of the Department of Parasitology, London School of Hygiene and Tropical Medicine and I am much indebted to Dr. R. T. Leiper, C.M.G., F.R.S., who allowed me to examine them and who gave me considerable help and encouragement throughout the work.

Viannella africana n.sp.

Host—*Thryonomys swinderianus,* a Cane Rat.

Position—Small Intestine.

Distribution—Southern Rhodesia, Kenya.

These nematodes were in a tube labelled "Trichostrongylids from a cane rat, S. Rhodesia". There were 23 worms which on examination seemed to approach in structure the Viannaiinae. Later two mounted specimens taken from a cane rat, which had died soon after admission to the gardens of the London Zoological Society, were found to have the same morphological structure as those from S. Rhodesia. The mounted specimens came from rats from Kenya. As this species does not seem to be identical with any yet described, an account of it is given below and the name *Viannella africana* is given to indicate its geographical distribution.

None of the worms was seen alive. Fixed in hot 70% alcohol and preserved in glycerin, they appear as relatively sturdy worms, rather dark in colour and generally coiled in 2 or 3 spirals, though a few had only a single spiral. They were not easy to clear because of the overlapping and it was necessary to use equal parts of phenol and lactophenol to make them reasonably transparent. The cuticle is much thickened and bears longitudinal lines crossed by fine transverse striations. These do not however extend to the extremities, the head and tail being covered with normally thin cuticle.

The head is somewhat inflated and marked off by an annular constriction about 62μ from the anterior end. This region is also marked by 4 or 5 rings formed by shallow constrictions which do not however involve the whole thickness of the cuticle and which may be

due to contraction. Cervical papillae were not seen. The mouth is terminal, a simple pore leading directly into the muscular oesophagus. The intestine is uninteresting and opens to the exterior by the usual chitinized rectum.

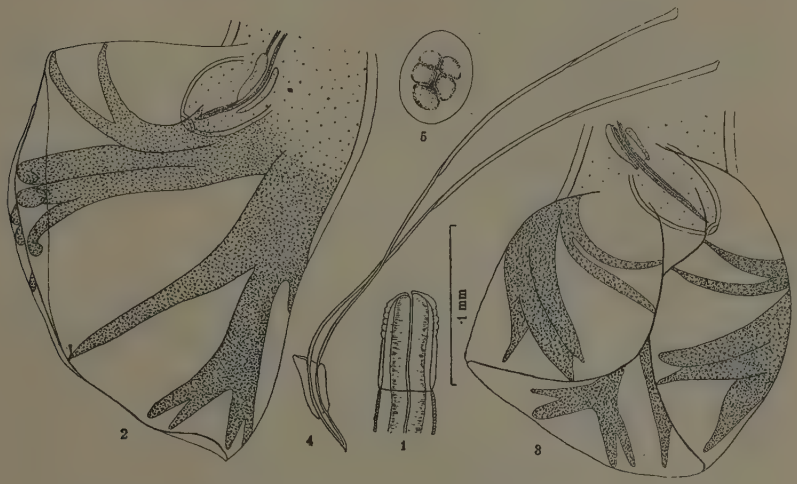
The female worm measures from 6.52-8.5mm. long. The tail is short, narrows sharply behind the anus and ends in a blunt point. The anus is a transverse slit 50-60μ from the posterior end while the vulval opening is placed about 100μ in front. The worm is monodelphic. The vulva opens through a short vagina into a large muscular ejaculatory apparatus, the posterior part of which is short with a thick wall formed by a dense mass of fibres, spirally arranged. It is preceded by a longer muscular portion which may measure up to 165μ long. The eggs lie in a single row in the uterus. They are large, have a thin shell and are apparently segmenting when passed. They are bluntly oval in outline and measure from 69-82μ long by 43-47μ broad.

The males are smaller than the females measuring only from 4.73-6.5mm. long They tend to be less tightly coiled than do the females. The bursa is large and well developed with thick fleshy lobes, which are slightly asymmetrical, the left lobe tending to be a little larger than the right. There is no distinct dorsal lobe. The ventral rays arise as a common trunk but diverge widely distally though both turn anteriad. They reach the tip of the bursa and sometimes seem to carry the membrane with them to form a slight prominence. The laterals also arise in a common trunk though the antero-lateral soon separates but continues to run in close apposition to the medio-lateral. Both are thick fleshy rays. The postero-lateral is short and arises as a branch of the medio-lateral. All these rays taper distally and the medio-laterals pass right to the edge of the bursa. The dorsals all arise in a common trunk. The externo-dorsals branch off and diverge widely but do not reach the edge of the bursa. The median unpaired dorsal ray bifurcates and its branches sub-divide with the inner branch slightly larger than the outer. None of the branches reaches the edge of the bursa. The bursal membrane is thick and fleshy and shows markings arranged in linear rows running roughly parallel with the rays.

The spicules are long slender structures, not easily focussed, owing to their structure. They are lightly chitinized rods, slightly unequal in length and measure from 385-395μ long. Each is twisted in a loose spiral. Proximally they widen to about 10μ wide but distally they are narrow while the tips are fused for a distance of about 7μ.

There is a gubernaculum formed of an ovoid plate rolled at the sides to form two gutters in which the spicules slide. It is only lightly chitinized. There is a large genital cone, without chitinous armature.

The relationships of this worm are not altogether clear. The possession of a single uterus places it within the Heligomosomidae and it can be excluded from the Heligmosominae by the presence of a well developed dorsal ray and by the spiral coiling. As the lateral lobes of the bursa are fairly evenly developed it falls readily into the subfamily Viannaiinae as defined by Neveu-Lemaire in 1934. The



Viannella africana.—Fig. 1. Anterior end. Fig 2. Bursa, lateral view. Fig. 3. Bursa, ventral view. Fig. 4. Spicules and gubernaculum. Fig. 5. Ovum.

formula and disposition of the bursal rays is typical of the genus *Viannella* and the hosts of all the species so far described are all rodents, though the distribution is purely South American. There are however important differences. The spicules within this genus are typical for each species but agree in being short and sturdy and there is no gubernaculum. The genital cone is generally armed. There are resemblances to the genus *Impalaia* particularly in the spicules which are long and slender and in the presence of a gubernaculum. But the differences are marked especially in the origin of the ventral rays and

in the form of the dorsal ray. Though the geographical distribution of the genus *Impalaia* is purely African, yet the hosts are all ungulates and the worms themselves are not spirally coiled. This spiral coiling is a character which has been considered as of some importance by most workers in this field and certainly the spiralled worms seem to form a homogeneous group.

Though its generic identification is a little confusing, it has been decided to include it, temporarily at any rate, among the species of *Viannella* on account of its bursal structure, the spiral coiling of the body and the rodent host. In order to do so however, it becomes necessary to emend the generic description with regard to the male. It must now be said that the spicules are generally short but may be long and that a gubernaculum may be present while the armature on the genital cone may be absent.

Cheilospirura falconis n.sp.

Host—*Falco tinnunculus*.

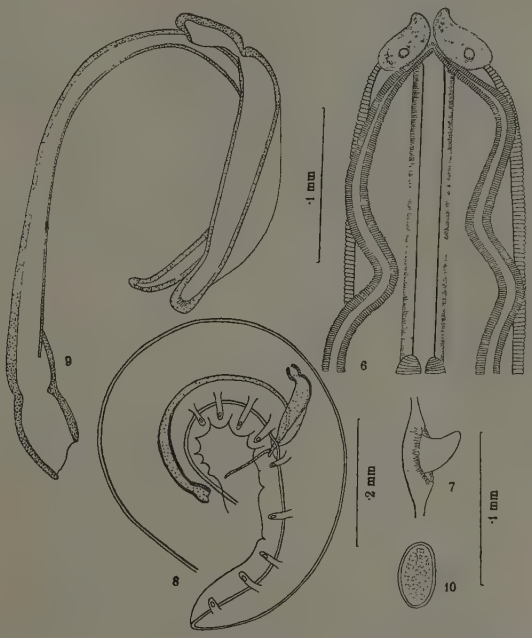
Position—Oesophagus, crop.

Distribution—Palestine.

The material consisted of part of the oesophagus and crop of a kestrel, *Falco tinnunculus*, in the tissues of which were firmly embedded the anterior ends of some nematodes. At least one third of the worms was so embedded and it was necessary to dissect them out. In both sexes the mouth is a simple pore surrounded by a pair of large fleshy lips drawn out anteriorly into prominent projections; each carries a pair of papillae. The cordons arise in pairs between the lips, separate immediately and follow a wavy course for about 2.1-2.5mm., without anastomosing or recurring. They are striated laterally and appear to have a median gutter, the total width being about 18μ in the widest part. The body cuticle is comparatively thick and is transversely striated. The cervical papillae are strong hook-like structures, outstanding prominently from the cuticle a short distance behind the first oesophagus. They are embedded in thick cuticle. The mouth pore opens into a cylindrical buccal cavity measuring about 200μ long. The wall is thick and appears to be transversely striated.

Only one undamaged female was available; it measured 14mm. long. The vulva lies behind the middle of the body dividing it into two portions in the ratio of 4 : 3. It is not salient. The tail is blunt

and measures 130μ long. This intact female and several others which were damaged, were all gravid with the uterus filled with a great mass of eggs which were embryonating. These measure from 37-43μ long by 21-28μ broad, are bluntly oval in outline with parallel sides and have a thick shell. The contents do not entirely fill the cavity but are centrally placed, their contour following the outline of the shell.



Cheilospirura falconis.—Fig. 6. Anterior end. Fig. 7. Cervical papilla. Fig 8. Posterior end of male. Fig. 9. Spicules. Fig. 10. Ovum.

The males varied in length from 9-11mm. The tail is long and tapering and measures from 122-138μ; it is permanently rolled into a spiral. The caudal alae are wide and striated. They are supported by 4 pairs of pre-cloacal papillae and by 5 pairs of post-cloacal ones. These are all stalked and the posterior ones tend to arise asymmetrically. The spicules are unequal and dissimilar. The right one is thick and sturdy with the anterior end sharply turned at a right angle and

with a ventral wing. It measures from 200-234μ long and terminates bluntly. The left one is long and slender, tapering distally. Its length varies from 485-520μ.

This species appears to differ fundamentally from others of the genus *Cheilospirura*. The size and arrangement of the caudal papillae is distinctive as are the total body measurements of the female. Furthermore in *C. hamulosa* the cordons extend for a long way down the body and in this species, their extent is limited. The body length of *C. rotundata* is somewhat similar to that of the present species but the position of the vulva in the posterior portion of the body differentiates it as does the size of the spicules. *C. spinosa* is distinguished by the nature of the cordons which consist of discrete sharply pointed elements and by the length of the spicule.

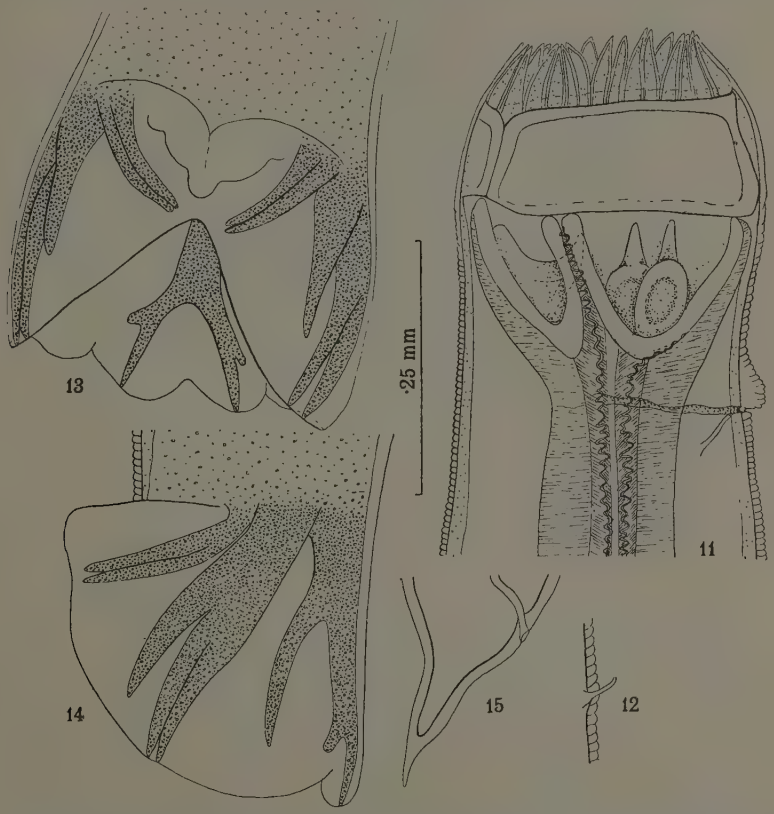
It would appear that this material does not closely agree with any yet described for this genus and the name *falconis* is given to indicate the type of host in which it may be found. The material was originally sent to this Institute by Major T. A. Cockburn to whom we are much indebted.

As a postscript to this section it may be recorded here that some material from a chicken in India has been provisionally described as *C. hamulosa* though the relative lengths of the spicules are anomalous. The ratio of the lengths is usually given as 6 : 1 and this has been confirmed by examination of considerable quantities of material in our collection. In this particular tube however, the ratio is 10 : 1, the longer spicule being much elongated. This proportion was constant throughout all the worms.

Oesophagostomum tridentatum Maplestone, 1932.

This species was originally described by Maplestone in 1932 who found it on three occasions in the stomach of a Dusky langur, *Semnopithecus obscurus* in India. Some nematodes examined by the present writer from the stomach of *Nasalis larvatus* from Borneo, appear to belong to this species. Certain differences are however apparent which seem worthy of record. The total lengths and general proportions agree closely with those recorded by Maplestone. The bursa too shows no differences. Maplestone describes the external leaf crown however as consisting of 20 broad pointed elements projecting beyond the mouth collar. In the Borneo material, the leaf crown consists of 30 such elements and this number was constant through all the nematodes.

The most striking differences however can be seen in the chitinization of the oesophageal infundibulum. This is hemispherical in shape with thick walls. The chitinization seems however to be laid down in three sections, one being dorsal and the other two latero-ventral. A tooth



Oesophagostomum tridentatum. Fig. 11. Anterior end showing chitinization of the buccal cavity and oesophageal infundibulum. Fig. 12. Cervical papilla. Fig. 13. Bursa, ventral view. Fig. 14. Bursa, lateral view. Fig. 15. Tail of female.

arises in each section, each consisting of a firm oval base and a forwardly directed crown which reaches nearly to the level of the buccal capsule. These sections are very obvious both in lateral and end-on

views and pressure on the cover slip causes them to separate. There are three cells at the base of the oesophagus, which are presumably glandular, as ducts run forward from them, following a wavy course, to open between these three sections of chitinization. Another small point of difference is that the external cuticle is coarsely striated.

References.

Cram, E. B., 1927.—" Bird Parasites of the Nematode suborders Strongylata, Ascaridata, and Spirurata." *No.* 140 *Bull. U.S. nat. Mus.*, 465pp. (W. L. 5659).

Maplestone, P. A., 1932.—" Parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens." *Rec. Indian Mus.*, xxxiv (3), 229–262. (W. L. 17746).

Travassos, L., 1921.—" Contribuicoes para o conhecimento da fauna helmintolojica brasileira. xiii. Ensaio monografico da familia Trichostrongylidae Leiper, 1909." *Mem. Inst. Osw. Cruz*, xiii (1), 82pp. (W. L. 13465).

1937.—" Revisao da familia Trichostrongylidae Leiper, 1912." *Monographias do Inst. Osw. Cruz*, No. 1, 512 pp.

Journal of Helminthology, Vol. XXII, No. 1, 1947, *pp.* 37—46.

On the identification of some species of *Trichostrongylus*.

By Phyllis A. Clapham, D.Sc.

(*From the Institute of Agricultural Parasitology, St. Albans*).

The spicules of the genus *Trichostrongylus* are the most important distinguishing feature of the various species. They have however a very complicated structure, being heavily ridged and twisted and because of this are not easy to interpret.

As nematode cuticle and internal tissue are readily dissolved by a number of chemical substances, Professor R. T. Leiper suggested from some preliminary experiments a method whereby they could be liberated from the surrounding tissues when they could be seen free from débris and rotated to facilitate investigation.

Experiment showed that NaOH was particularly efficient for this purpose and that it needs no special technique. At laboratory temperatures the process is too slow to be of much value for diagnostic purposes but in an oven at 37°C., complete solution is achieved rapidly and successfully. Fresh unfixed trichostrongyles react quickly; 1% or 2% solutions caused rupture of the cuticle in 2 hours and when left all night, all the soft tissues had disappeared and the spicules themselves had been acted upon with resulting darkening and distortion; 3% solution frees the spicules within 2 hours; the same end is reached in 45 minutes when 4% or 5% solutions are used.

Treatment with NaOH at laboratory temperatures produces much slower results. Solutions under 3% seem to have no solvent action, but worms can be left overnight in 3%, 4% or 5% solutions without fear of distortion. Most of the spicules will be found to be free when the dishes are examined next morning.

Fixed material reacts more slowly than fresh. In high dilutions, *e.g.* 1% or 2%, solution is very slow, even at a temperature of 37°C, there being scarcely any change after immersion for 4 days. Worms can however be left in 4% or 5% solutions in an oven for 12 hours quite safely; most spicules will have been freed in 6 hours and a few will still be lightly surrounded by tissue even after immersion overnight in most species. There is however some difference between the reactions of different species and even within species, and the duration and method of fixation seems to have a slight effect on the rate of solution. It is therefore necessary to experiment a little with each batch of material.

All freed spicules are eventually affected by prolonged immersion in the caustic alkali. They begin to darken, later become swollen in their thin parts and finally are dissolved completely. It is necessary therefore to remove them to a neutral medium before distortion begins, when they are needed for morphological examination. The most practical method of isolation was found to be as follows :—the selected worms are put into a 4% solution of NaOH in a solid watch glass under a cover to prevent evaporation, in an incubator at 37°C. This is done early in the morning if the material is fresh but last thing at night if it is fixed. When the internal tissues are dissolved but before the cuticle has finally disappeared, the alkali is neutralized with a few drops of 5% HCl. Transference to another watch glass or a slide by means of a fine micro-pipette induces sufficient traction to suck them out of this covering and when carefully done often leaves the spicules in pairs with the gubernaculum so that it is easy to know when individual pairs of spicules are being examined. By neutralizing the alkali, the process of solution is stopped and they can be examined more or less at leisure within the next day or two; decomposition processes set in later unless special precautions are taken to prevent them. Freed spicules are not easy to handle for they are much softened by the treatment and rough handling causes them to buckle and bend. They can however be safely picked up with a fine pipette and can even be rolled under a cover slip if plenty of water is present. Treatment with formalin or spirit will not harden them.

As the material available was usually a mixture of species of *Trichostrongylus* with *Nematodirus, Ostertagia* and *Haemonchus,* some observations were also made on these genera. The cuticle of species of *Nematodirus* seems to be more resistant and to take longer to break down than does that of *Trichostrongylus,* while the reverse is true of *Ostertagia* species. The spicules however of *Nematodirus* are readily affected by the alkali and so far it has not been found possible to separate them in a condition satisfactory for examination. They are

Trichostrongylus axei

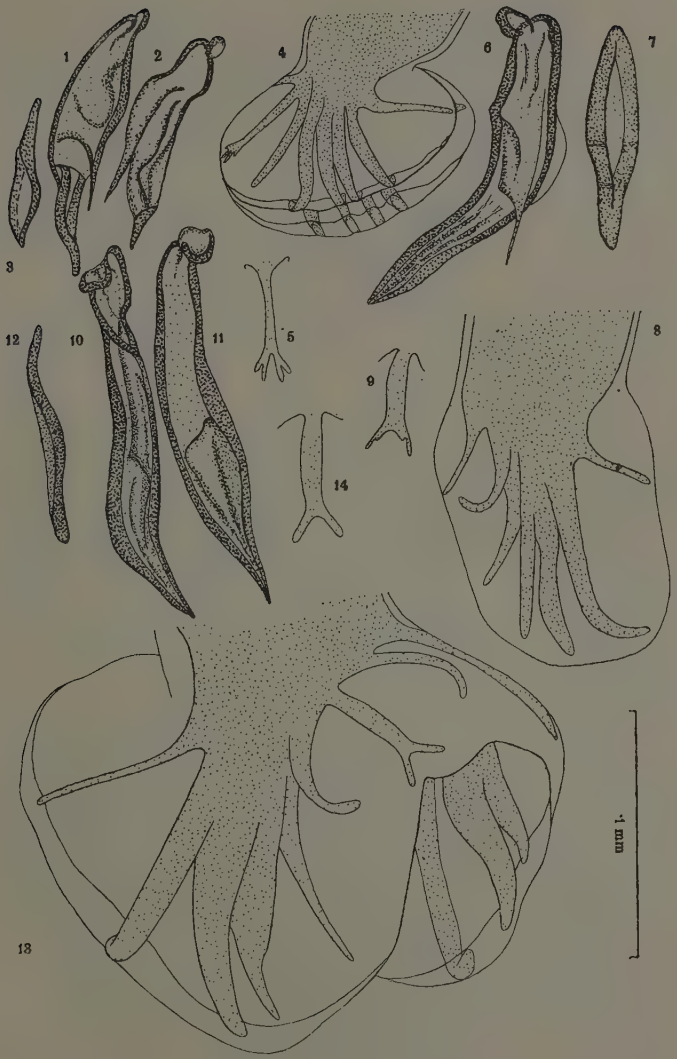
Fig. 1 Left spicule. Fig. 2 Right spicule. Fig. 3 Gubernaculum. Fig. 4 Bursa. Fig. 5 Dorsal ray.

Trichostrongylus capricola

Fig. 6 Spicule. Fig. 7 Gubernaculum. Fig. 8 Bursa. Fig. 9 Dorsal ray.

Trichostrongylus vitrinus

Figs. 10 & 11 Spicule—lateral and ventral views. Fig. 12 Gubernaculum. Fig. 13 Bursa. Fig. 14 Dorsal ray.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
1 mm

invariably blackened and shrunken before they are released. The species of *Ostertagia* however stand up well to this treatment. They are quickly and easily freed and are not readily distorted unless left for several hours in the solvent. Comparison of artificially freed spicules of *Ostertagia* with those still enclosed and with the figures published by Travassos show very exact agreement, suggesting that this treatment is suitable for their examination.

This technique has been used with other cuticle solvents but none have given such systematically good results as NaOH. KOH is useful but the action is more rapid than that of NaOH in similar dilutions and direct action on the spicules begins early, leading to distortion. Hence it is necessary to watch the process at short intervals which is in itself a disadvantage as one is not left free for other occupations, while the reaction goes on.

The sulphides K_2S and Na_2S were also used but unsatisfactory results were obtained. The reaction seems to proceed rapidly up to a certain point and then stops. The quantity of free H_2S is much reduced by this time and may be the cause but addition of fresh sulphide does not succeed in completely finishing the process, though solution does proceed very slowly for some time. No really satisfactory results were ever obtained with these substances and they were discarded as suitable solvents.

Examination of a number of species of *Trichostrongylus* by this method has revealed some new features in the morphological structure of the spicules. The bursa has also been re-examined using the normal methods of clearing.

Trichostrongylus axei has spicules which are markedly unequal in size and shape. The right one is the smaller and measures, in material from sheep, from 82-100μ long. The left one measures from 110-115μ long and ends in a long curved point. Both have an ear-like projection anteriorly and both give off medially a thin chitinous wing which is drawn out to a sharp point. On the small spicule this has apparently been noticed before and appears in the text descriptions but it is not

Trichostrongylus colubriformis

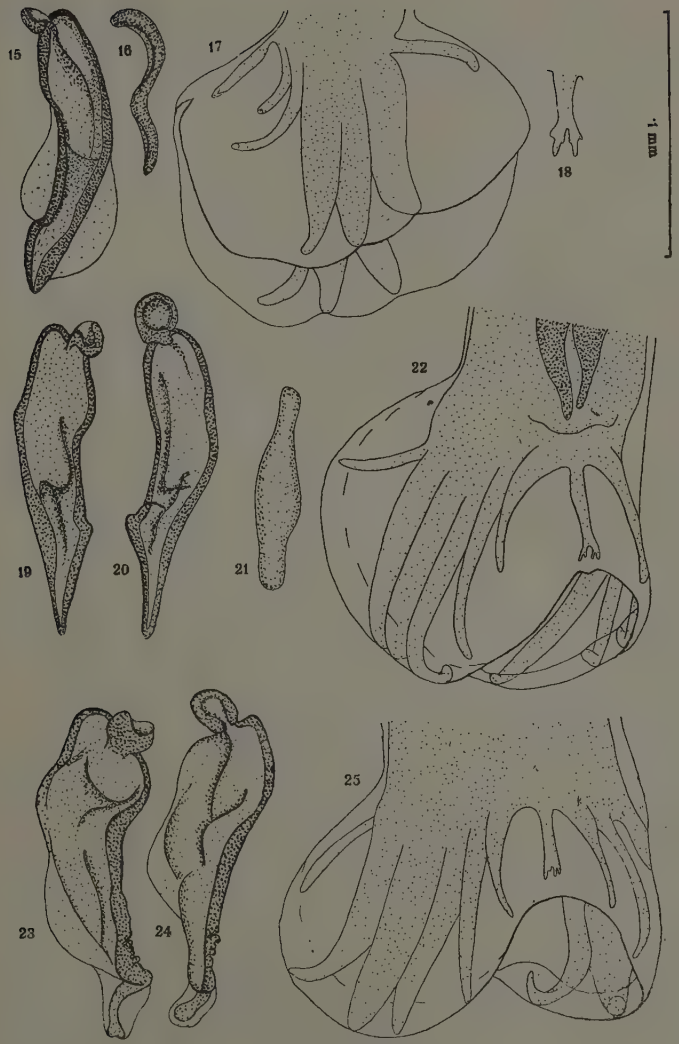
Fig. 15 Spicule. Fig. 16 Gubernaculum. Fig. 17 Bursa. Fig. 18 Dorsal ray.

Trichostrongylus falculatus

Fig. 19 Right spicule. Fig. 20 Left spicule. Fig. 21 Gubernaculum. Fig. 22 Bursa.

Trichostrongylus rugatus

Fig. 23 Right spicule. Fig. 24 Left spicule. Fig. 25 Bursa.


15
16
17
18
1 mm
19
20
21
22
23
24
25

featured in any of the drawings. It is however an obvious feature of the spicule and absence of it in published drawings is liable to lead to misunderstandings. *In situ* these wings overlap. The gubernaculum is more lightly chitinized and measures 50-65μ long.

The bursa is small. The antero-lateral rays are the stoutest but are closely approached in size by the latero-ventrals and the medio-laterals. The dorsal ray is slender, bifurcate and each branch divides again. The antero- and medio-laterals lie roughly parallel to each other while the ventro-ventrals and ventro-laterals turn forward. There is no distinct dorsal lobe. The bursa often lies partly opened and its structure is not difficult to follow.

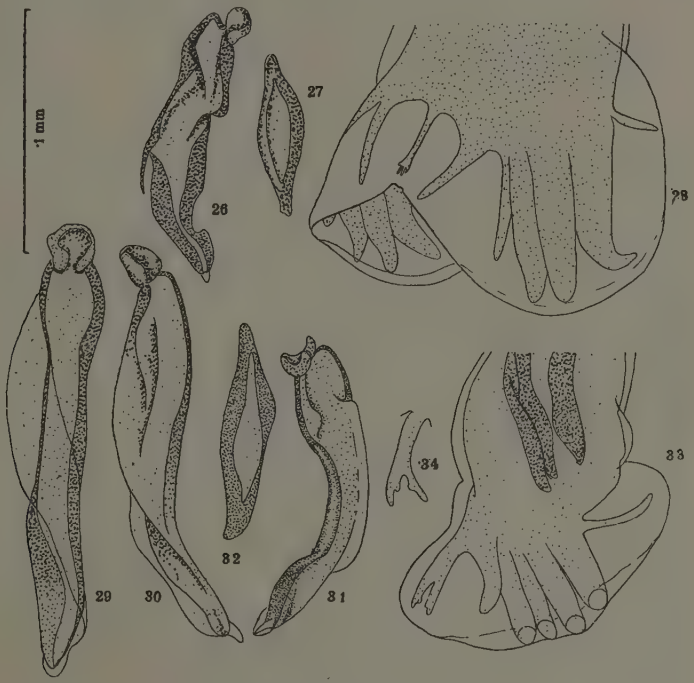
Trichostrongylus capricola. The spicules are equal and similar and simple in form. There is a lateral wing drawn out to a fine point which does not seem to have been described before and which is very difficult to focus in position in the worm. It is so fine that it is one of the first parts of the spicule to be attacked by the alkali. A very thin semi-circular wing arising dorsally is also present. The spicules measure from 135-148μ long, are bent at an obtuse angle and taper to a point distally. There is an ear-like appendage anteriorly. The gubernaculum is broad and measures from 90-99μ long.

The bursa is elongate. The latero-ventrals are long and sturdy with the ends turned ventrally. The antero-laterals are also sturdy and turn slightly. The dorsal ray reaches almost to the edge of the bursa, bifurcates and each branch divides again but the two inner branches remain stumpy while the outer ones are longer and slender.

Trichostrongylus vitrinus has equal and similar spicules which superficially resemble those of *T. capricola.* They can be distinguished however by their lack of wings drawn out to a point, by their sudden tapering to a very sharp point distally and by their comparative straightness as well as by their larger size. They vary in length from 150-170μ. Complex ridging and twisting seem to be absent. The gubernaculum is spatulate in surface view, boat-shaped in the lateral view which also shows considerable curvature. It measures 88-90μ long.

The bursa is often fairly wide open with the edges of the lobes turned in. The externo- and medio-lateral rays are sturdy and run parallel, while the latero-ventrals diverge sharply from them. The dorsal ray bifurcates and each branch remains intact. There is no distinct dorsal lobe.

Trichostrongylus retortaeformis has short stumpy spicules, equal and similar in size, with an ear-like projection anteriorly. They are broadest about half way down and a fine chitinous point takes origin behind the middle where the spicule begins to narrow. Distally they



Trichostrongylus retortaeformis

Fig. 26 Spicule. Fig. 27 Gubernaculum. Fig. 28 Bursa.

Trichostrongylus tenuis

Figs. 29 & 30 Left spicule—two views. Fig. 31 Right spicule. Fig. 32 Gubernaculum. Fig. 33 Bursa. Fig. 34 Dorsal ray.

broaden again to form a shape something like an ill-formed ploughshare which is abruptly pointed. The distal portion of the spicule is bent at an acute angle. The total length of the spicule may be as great as 129μ which is greater than the published figures. The gubernaculum measures up to 72μ long and has the typical boat shape.

The bursa is fairly small and is often closed so that a little manipulation may be necessary before its rays become quite visible. There is no dorsal lobe. The dorsal ray is bifurcate and each branch divides again to form 4 short points. On each side the externo-dorsal and postero-lateral rays diverge widely. The medio- and antero-lateral rays run parallel and the latero-ventral is parallel for some distance but turns forward at its tip. The stoutest rays are the antero-laterals and the latero-ventrals.

Trichostrongylus rugatus. Only one male was available for examination. Hence solution of the soft parts and consequent destruction of the worm was not feasible. The spicules are dissimilar in shape and much stouter than are those of other species examined. The larger one measures 147μ long, is broad anteriorly but the posterior half tapers regularly and ends in a blunt process set at right angles to the main body. There is an irregular area of thin cuticle surrounding this process. The smaller spicule measures only 136μ long and is about 33μ wide for nearly all its length but tapers suddenly to form a blunt, slightly bilobed process set at an acute angle. This process is also surrounded by thin cuticle. Though the spicule is practically the same width during its whole length, yet in the lateral region it is very thin. Medially the cuticle is much ridged and puckered in front of the blunt distal processes. There is the usual ear-like projection anteriorly.

The bursa was closed with the lobes closely folded and over-lapping. The dorsal ray is bifurcate and each branch subdivides but the sides do not remain symmetrical. On the left side the branches are equal in length but on the right side the inner branch appears to be curtailed. The antero-lateral rays are the thickest and they run parallel with the medio-laterals for most of their length though the tips diverge. The latero-ventrals are thick and turn in their distal half. None reaches the edge of the lobes. There is no distinct dorsal lobe.

Trichostrongylus falculatus has dissimilar spicules. The larger one measures from 136-145μ long. Anteriorly there is the typical projection. The spicule is fairly simple in structure with little torsion and ridging; there is a thickening extending about half the length which overhangs the edge, but which is not drawn out to a point. The main body of the spicule terminates in a blunt triangular shape, concave on one side. The smaller spicule measures from 125-132μ long and is relatively stouter proximally than the other. It tapers distally to a

blunt point but the sides remain fairly straight with scarcely any concavity. The gubernaculum measures from 82-85μ long, is spatulate and only very lightly chitinized.

The bursa is easy to follow as the rays are not very dense. There is no distinct dorsal lobe. The dorsal ray is bifurcate and each branch divides again into 4 equal branches. The antero-lateral rays are the thickest but they are closely approached in size by the latero-ventrals. These two and the medio-laterals run parallel and are in close apposition to each other.

Trichostrongylus tenuis has complex spicules which are difficult to follow as they are much twisted. They are somewhat dissimilar both in size and shape. In the material investigated here, which was all obtained from partridges, the range is greater than is usually given in publications. The left one varied in size from 126-183μ long and the right from 100-164μ long. The left one is gently curved. There is a wide wing of cuticle arising shortly behind the anterior end which passes spirally round the body and encloses the tip. The right spicule has a much stronger curvature and medially is deeply grooved. Towards the distal end the sides of this groove are raised to form a semilunar shape. This spicule is sharply contracted in the anterior region and the spiral cuticular wing is less strongly developed than in the larger spicule. The gubernaculum shows some variation in shape being roughly spindle shaped and elongated anteriorly. The broad central region has an asymmetric anterior thickening. It is heavily chitinized particularly along the margins and measures from 70-87.5μ long.

The bursa is supported by well developed rays of which the latero-ventrals are very stout and the antero-laterals nearly as stout. These two, with the medio- and postero-laterals lie closely parallel along their whole length. The externo-dorsals are well developed and turn forward at their tips. The ventro-ventrals lie at right angles with the others and point forward. The dorsal ray is sturdy, bifurcate and each branch subdivides again into an inner stumpy and an outer elongated branch, but there is considerable variation between individuals in this character.

No other species of *Trichostrongylus* were available for examination.

The basic plan of the spicule in this genus seems to be as follows :— The main body is strong, ridged, twisted distally and tapers to a point. Anteriorly there is a well chitinized projection for the insertion of

muscles. Medially are given off cuticular wings one of which is frequently elongated into a fine point or drawn out to a blunt projection overhanging the side; others retain a rounded outline. All kinds of variations occur on this plan. The torsion of the body may be slight as in *T. vitrinus* or pronounced as in *T. tenuis*. The degree of ridging or thickening varies considerably but it is usually marked. The cuticular wings may be extensive as in *T. tenuis* or reduced as in *T. axei*. The distal region may be pointed but is often broadened out into an expansion in the shape of a ploughshare. The gubernaculum is typically boat-shaped in lateral view, spatulate in surface view, and well chitinized. There is not much variation in this typical design.

Summary.

1. A method is described by means of which trichostrongylid spicules are separated from the surrounding soft tissues and their examination made easier. NaOH and KOH are the most satisfactory substances for this purpose, though both substances eventually act on the chitin of the spicules themselves, causing distortion. The reaction takes place at a temperature of 37°C. with reasonable speed. Spicules of *Ostertagia* can also be freed satisfactorily by this method but those of *Nematodirus* are quickly affected by the alkali, with consequent distortion.

2. The spicules and bursae of 7 species of *Trichostrongylus* have been examined and certain new points have come to light.

Journal of Helminthology, Vol. XXII, No. 1, 1947, *pp.* 47—48.

On the occurrence of *Davainea madagascariensis* on the African mainland.

By Phyllis A. Clapham, D.Sc.

(*From the Institute of Agricultural Parasitology, St. Albans*).

Davainea madagascariensis is a rather rare human parasite, there being less than a dozen cases on record. It was originally described from children in the Comoro Islands by Davaine in 1869 under the name *Taenia madagascariensis* and has since been recorded from a wider area stretching from Madagascar and the neighbouring islands through Siam to the Philippine Islands in the Pacific Ocean. Recently some cestode segments were sent to Dr. R. T. Leiper, C.M.G., F.R.S. They had been passed in the stool of a human patient in S.E. Africa. They have now been identified as gravid segments of *Davainea madagascariensis* and this would seem to be the first record of the species from the mainland of Africa.

The segments are thick fleshy structures 1.3mm. broad by 1.6mm. long, rather dark in colour but as they arrived fixed in alcohol this fleshiness and colour may have been due, in part at least, to contraction in fixation. Anteriorly they are markedly convex while posteriorly they are concave with a deep groove into which the following segment is attached. The genitalia have completely disappeared and the contents consist of a mass of parenchyma in which are embedded over 150 egg capsules, delimited by thick fibrous walls. Each capsule contains 3 ova, elongated in shape to a length of 55-60μ while the breadth remains in the neighbourhood of 20-24μ. The contained onchospheres are spherical and measure 12μ in diameter. Embryo hooks measuring 4-5μ long could be seen in most though it was generally difficult to see the full complement of three pairs in all.

There has been some discussion in the past as to whether *Davainea madagascariensis* should also include the material described by Daniels in 1895 from a human patient in British Guiana and called by him *Taenia demerariensis* but which is now recognised as a species of the genus *Davainea*. Joyeux & Baer, who were able to examine a quantity of this material, came to the conclusion that though very similar, it represents a distinct species, one of the distinctions being seen in the gravid segments. In material from the Old World the capsules in

these segments contain 2-3 ova only while in that from the New World there is invariably a larger number, ranging from 6-10. They noted other differences in the strobila. On these grounds, the present material must be identified as *D. madagascariensis*.

References.

Daniels, C. W., 1895.—" *Taenia demerariensis*." *Brit. Guiana med. Annu.*, 7, 95–98. (*W.L.* 3556).

Joyéux, C. & Baer, J. G., 1929.—" Les cestodes rares de l'homme." *Bull. Soc. Path. exot.*, 22 (2), 114–136. (*W.L.* 5310).

Journal of Helminthology, Vol. XXII, No. 1, 1947, pp. 49—60.

The Incidence of *Trichinella spiralis* at Necropsies in England.

May R. Young, B.Sc.

(*University College, Nottingham*).

Our knowledge of *Trichinella spiralis* (Owen, 1835) infection in man in Great Britain can be divided into (a) clinical cases reported before 1941, (b) those reported since and (c) infections diagnosed by examination of necropsy material. Prior to the outbreaks in Wolverhampton, Harpenden, Penrith, Birmingham, Lewisham and Greenwich (in chronological order) in 1941 only 134 clinical cases had been reported in Britain. Of these 77 were in widely separated localities in England and 57 were in Wales.

The first outbreak to be recognised in Britain was at Workington in 1871 when the illness of three persons was shown to have been caused by their having eaten the flesh of a home-bred sow (Dickinson, 1871 and Cobbold, 1871). The second outbreak in 1879 involved 44 cases at Thaxted, Essex (Armistead, 1879). From that time until 1941 most of the reported cases have been sporadic. They number 31 in England and 57 in Wales. One of these was fatal (Brailsford, 1926).

As the cardinal symptoms of trichinosis *viz.* the swelling of the eyelids and muscular pain, depend largely on the number of infective larvae swallowed, microscopic examination of necropsy material has been found to be the most reliable method of determining the percentage incidence of infection. The early trichinosis statistics in Britain were the result of naked-eye observations by anatomists in Edinburgh. In 1843 Knox reported 3 out of 500 cadavers having minute white calcified *Trichinella* cysts, and in 1860 Turner reported 6 positives and stated that he had found *Trichinella* in 1-2% of all cadavers he had occasion to examine during five years. In 1937 van Someren found 1% of 200 human diaphragms of persons dying from miscellaneous causes in London hospitals positive by the artificial gastric juice or "digest" method of detection. In 1941 an opportunity for using this method was afforded by an outbreak at Wolverhampton. In May of that year the result of the examination of 47 human diaphragms from several English towns was given in a discussion at a meeting of the Royal

Society of Medicine (Young in Beeson, 1941). Five out of 30 diaphragms from Wolverhampton and one out of 5 from Birmingham were positive. Two of the Wolverhampton infections showed heavily calcified cysts of several, if not of many, years' standing. Human diaphragms from Cambridge, Bristol, Cardiff, Llandough and Leeds have since been examined.

Method.

The diagnosis technique used when examining pork was by direct examination of three small pieces of a specimen from the pillar of the diaphragm. These were pressed between two slides and examined under the microscope. After the first 1,000 specimens were found to be negative the remainder of each specimen was kept and examined in batches by the artificial gastric juice or "digest" method. Each batch of specimens from one locality was minced, weighed and placed in 0.5% pepsin and 0.7% hydrochloric acid solution at approximately 37°C. (using 10cc. of solution per gram of meat) and then incubated at 37°C. and stirred every hour during the day. This "digest" was incubated for from 36-48 hours, depending on the amount of digestion undergone in that time, and then poured through a 40-mesh per inch copper gauze sieve (average size of the interstices being 0.374mm.) into a funnel having a rubber tube and clip at the base, and left for several hours to sediment. The digest container was rinsed with warm water which was poured over the undigested material held back in the sieve to wash through any free larvae or cysts which might be adhering to it. The average weight of 1802, the last out of 4,626 pork specimens from Wolverhampton, was approximately 8 grammes and that of 589 pork specimens from the Argentine pigs was 16 grammes. This technique was also used for human diaphragms which were preserved by immersion for a short time in 3% formalin and subsequently "digested" for 24-36 hours. On the average 80 grammes of each cadaver were examined in this way. About 5 cc. of the sediment at the base of the funnel was run into a small dish and portions of the sediment were poured over a large slide (5″ × 1½″) forming a thin film which was examined microscopically. Larvae or cysts, non-calcified or calcified were obtained in this way. But with a view to finding out the degree of infection the sediment examined and the whole of the "digest" in the funnel was washed with warm water through a fine mesh copper gauze sieve (average size of interstices being 0.130mm.). The material

held back by the sieve was then washed and centrifuged, the sediment being examined for larvae and cysts under the microscope. If positive, the number of larvae or cysts per gramme of muscle was obtained from the count. The sediment from the base of the funnel was examined before washing the whole "digest" through the fine sieve in order to observe the nature of the cysts, *i.e.* thin-walled, thick-walled with polar calcification, or calcified throughout. Experience showed that if the specimen was digested longer than actually necessary to digest the muscle fibres away from around the cyst, the cyst wall itself began to be digested; and if very thin, the mechanical disturbance of washing against the mesh of the sieve liberated many larvae from the cyst walls.

The sizes of the interstices of the sieve were measured and compared with the diameter of the coils of the larvae and the diameter and length of cysts of varying ages. The sieve through which the larvae and cysts were intended to pass had interstices averaging 0.374mm. The average length and diameter of the cysts was 0.348mm. and 0.313mm. respectively, and the average diameter of the coils of the larvae 0.221mm. The fine mesh sieve had interstices averaging 0.130mm. in size, and it will be seen that the measurements of the *Trichinella spiralis* cysts and coils of the larvae come between those of the interstices of the sieves, the one sieve allowing them to pass through and the other holding them back. The average diameter of the larvae was found to be 0.036mm. so that if the larvae happened to be stretched out and washed at right angles to the mesh of the fine sieve, they would pass through the interstices of 0.130mm. easily. In the "digest" the live larvae are coiled, as they are within the cyst, so that the fine mesh sieve is calculated to hold them back. Moreover, as there are varying quantities of undigested muscle fibres and connective tissue which tend to block the sieve, it is not practicable to use a sieve of finer mesh. In addition, the larvae if free in the digest would necessarily be alive as they would have been derived from very recently acquired infections (unencysted) or fairly recent infections (thin-walled cysts), as it is not until the cyst wall is very thick and calcified that the larvae die. Thus, in a technique where the digest is allowed to proceed only so far as to digest muscle fibres and has been found to show thin-walled cysts containing live larvae, there is no risk of losing straightened-out dead larvae in the washing process through the fine sieve, as these are contained in thick-walled, usually calcified, cysts which are too large (diameter 0.313mm.) to pass through the interstices (0.130mm.).

Results of Examination of Human Diaphragms.

Altogether 51 or 10.81% of 472 human diaphragms from Wolverhampton, Birmingham, Cambridge, Bristol, Cardiff, Llandough and Leeds have been found to be infected with *Trichinella spiralis*. The infections were of all ages, some recently acquired *i.e.* unencysted larvae, some of several months' duration *i.e.* larvae within a thin-walled cyst, some of a year to several years' duration *i.e.* live larvae contained in thick-walled cysts with polar calcification, and some of many years' duration *i.e.* many-layered thick-walled calcified cysts containing amorphous remains of dead larvae. An average of less than one larva or cyst per gramme of human diaphragm was the most general degree of infection, a few diaphragms however averaged up to 8 larvae or cysts per gramme. The numbers of each of these types of infections from each locality are given in Table II. Where double infections have been present in one diaphragm the number has been added in brackets.

The incidence of *Trichinella spiralis* infections in human diaphragms has been tabulated according to sex and locality in Table I, and according to total incidence, locality and nature of infection in Table II.

Table I

Sex incidence of *Trichinella spiralis* in human diaphragms

Locality	Males				Females			
	Number exam'd	Positives			Number exam'd	Positives		
		No.	%	S.E. %		No.	%	S.E. %
Birmingham ..	122	16	13.1	3.05	72	8	11.1	3.70
Wolverhampton	66	8	12.1	4.02	40	8	20.0	6.35
Cambridge ..	19	2	10.5	7.00	18	0	0	—
Bristol ..	24	2	8.33	5.15	22	4	18.2	8.25
Cardiff ..	36	1	2.78	2.74	20	1	5.0	4.85
Llandough ..	5	0	0	—	2	1	50.0	35.4
Leeds	10	0	0	—	14	0	0	—
Totals ..	282	29	10.28	1.79	188	22	11.69	2.32

Assuming that the 472 cadavers represented in this survey can be regarded as a random sample of the population of England and Wales, then the average incidence of *Trichinella spiralis* infection in that population is estimated at 10.805% with a Standard Error of 1.429%. That

is to say, it is highly probable that the true incidence (of which 10.805% is taken as an unbiased estimate) lies between the limits 7.9% and 13.7%.

It would appear, from Table I, that (a) the incidence is slightly higher in females than in males over the country as a whole, and (b) in some towns it is, on the contrary, higher in males. Either or both of these indications may be true, but when the Standard Errors are taken into account it appears more likely that the apparent sex differences are merely random fluctuations. Thus, the general sex incidence from the totals of Table I, treated as a 2 × 2 contingency table, gives a value for chi-squared (corrected for continuity by Yates' method [see Fisher, R. A. and Yates, F., 1938]) of 0.111. From this it can be shown that if the incidence is really the same for the two sexes, a discrepancy as high as that observed would occur by chance in between 70% and 80% of similar examples.

It is therefore justifiable to consider the two sexes together, as in Table II. This has the advantage of roughly doubling the numbers available for considering differences between towns.

TABLE II.

Total incidence of *Trichinella spiralis* in human diaphragms

Locality	Males and Females				Nature of Larval Infection			
	Number exam'd	Positives			Un-encysted	Thin-walled cysts	Calcified cysts	Larvae dead in calcified cysts
		No.	%	S.E.%				
Birmingham ..	194	24	12.4	2.35	5(+2)	4	6	7(+2)
Wolverhampton	106	16	15.1	3.45	6(+1)	2	1(+1)	5(+2)
Cambridge ..	37	2	5.4	3.70	0	0	0	2
Bristol ..	48*	6	12.5	4.75	0(+2)	2	1(+1)	1(+1)
Cardiff ..	56	2	3.57	2.50	1	0	0	1
Llandough ..	7	1	14.3	13.2	0	1	0	0
Leeds	24	0	0	—	0	0	0	0
Total ..	472	51	10.805	1.429	12(+5)	9	8(+2)	16(+5)

* Includes 2 cases of unknown sex omitted from Table 1.

Here again, however, the differences are not significant when pairs of towns are compared (the numbers of positive cases in the low-incidence towns are too small to justify the use of the chi-squared test). The extreme case of Leeds can be considered from a different aspect, thus, supposing the incidence to be 11% in all towns, what is the

probability of a consecutive run of 24 negative results (as at Leeds)? The required value is $(0.89)^{24}$ which is 0.061. Thus in over 6% of towns one would expect a run of 24 negative results.

It is of interest that 7 out of 26 infections in Wolverhampton and 7 out of 18 in Birmingham showed unencysted larvae. These were probably acquired at the time of the 1941 outbreak or even since that time. The occurrence of thick-walled calcified cysts in cadavers from each town except Llandough and Leeds, from which very small numbers have been tested, indicates that *Trichinella spiralis* of man has been endemic in those areas for many years.

The presence of definite double infections shows that one infection does not render the person permanently immune.

As it might reasonably be expected that a higher percentage of infections would occur in the older age groups the percentage incidences have been tabulated in deceunia in Tables III and IV.

TABLE III.

Age incidence of *Trichinella spiralis* in human diaphragms

Age at death Years	Number of cases			% Positive			
	Negative	Positive	Total	A	B	C	D
15—24 ..	27	2	29	6.89%	6.89%		
25—34 ..	36	5	41	12.19%	10.00%	9.30%	
35—44 ..	54	5	59	8.47%			12.5%
45—54 ..	85	17	102	16.66%	12.67%	16.66%	
55—64 ..	101	10	111	9.00%			
65—74 ..	62	8	70	11.42%	14.11%	11.22%	11.2%
75—84 ..	11	4	15	26.66%			
Totals ..	376	51	427	11.94%			

(Information as to age was not available for 96 diaphragms not included in the above table, but it is presumed that these would be of random distribution in the age groups. Thus the table has no value other than for age incidence. None of those omitted was infected with *Trichinella*.)

A glance at the percentage values (column A) suggests that the age-groups are too narrow to give any useful information. If all but the first are combined in pairs (column B) there appears to be a definite trend, the incidence rising from 6.89% in the lowest age-group to 14.11% in the highest. The data as they stand are not suited to chi-squared analysis, containing as they do the low frequency 2 in the first group. If they are combined into three groups, (i) the first three, (ii)

the central group, (iii) the last three (column C), they suggest a central peak with lower extremes. Finally, if two groups are made by combining the first four and the last three (column D), it now appears that positives are *more* numerous in the young than in the old. Actually, grouping as in column C gives $X^2 = 3.093$ which, for two degrees of freedom, has a probability of between 20 and 30%. The difference of column D is not significant. A further grouping (not tabulated) of (i) first three age groups, (ii) fourth, (iii) fifth, and (iv) last two, gave $X^2 = 4.286$ which, for three degrees of freedom, has a probability of between 30 and 50%. It must be concluded that there is no evidence of any differential age incidence; the suggestion given by column B is quite spurious and depends on the chance run of the frequencies.

TABLE IV.

Probable duration of *Trichinella spiralis* infections in the age groups

Age at death Years	Number of diaphragms with :			% with thick-walled cysts
	Larvae and Thin-walled cysts	Thick-walled cysts	Total	
15—24	2	2	4	50.0
25—34	3	2	5	40.0
35—44	3	3	6	50.0
45—54	12	8	20	40.0
55—64	2	10	12	83.3
65—74	1	7	8	87.5
75—84	1	3	4	75.0
Totals	24	35	59	59.3%

Table IV shows an increase in the percentage incidence of thick-cysted infections of long standing with increasing age. The total number of infections (59) is greater than the total of positive cases (51—see Table III) because in six cadavers there was clear evidence of a double infection, larvae and/or thin- and thick-walled cysts having been recovered.

EXAMINATION OF PIG DIAPHRAGMS.

The 4,626 specimens of pork received from the Wolverhampton abattoir during 1941 and 1942 were all negative for *Trichinella spiralis*. More than half this number were from sows, boars and stags. During the three months following the outbreak early in 1941 a specimen from

the pillar of the diaphragm of every pig killed in the Wolverhampton abattoir was examined microscopically. After the first 1,000 specimens the "digest" technique was adopted (as described earlier in this paper). The average weight of the last 1,802 specimens examined was 8 grammes. Some of the pigs killed in Wolverhampton came from widely separated localities in England.

Examination of Imported Pork.

Argentine pork was suspected of having been responsible for the Greenwich outbreak and was tested by the "digest" technique. All 589 specimens averaging 16 grammes were negative.

Examination of Rat Diaphragms.

Direct microscopic examination of diaphragms of rats have revealed *Trichinella spiralis* in nine out of 116 rats in Penrith and two out of 145 rats from the Wolverhampton area. These latter were two out of 13 from a sewage farm (Leiper in Beeson, 1941). 24 rats from several localities in Pembrokeshire and three from a farm near Uckfield, Sussex were negative.

Discussion.

Though the 51 infections of *Trichinella spiralis* in human diaphragms from Southern England are light, nevertheless the incidence, 10.81% is much higher than was expected considering the relatively few outbreaks of trichinosis reported in this country compared with those in the United States of America. In that country over 4,000 cases with an average of 10% fatalities have been reported between 1842 and 1936, whilst the average incidence from necropsy material is 16.3% (489 : 3,000) Wright, 1939, and 16.3% (488 : 3,000) Kerr, Jacobs & Cuvillier, 1941, and the average incidence resulting from 21 surveys, some of which were made by microscopic examination only, 14.0% (744 : 5,531) Wright, 1941. Lewis *et al,* 1940, applied correction for the differences in technique used in the surveys and stated that the incidence then in America was between 17% and 18%. This is closer to the incidence of 16.1% (855 : 5,313) quoted by Wright, Kerr & Jacobs, 1943. The present writer suggests that the incidence in England, higher than expected in comparison, may be due to the sieving technique used which enabled very light "dead" infections to

be identified. That the *Trichinella* incidence figure ought to be considerably lower in Southern England as compared with America is also shown by the infrequency of the infection in home-bred pigs, none having been found in the present survey nor by Hardy, 1927, or Howell, 1941; two definite instances only being known so far, *viz.* at Workington by Dickinson, 1871, and near Exeter by Harris, 1909.

Comparable incidence figures are not obtainable for the countries of Europe. Available figures have been quoted by Badel, 1933, and by Hall, 1938. The latter author states that over the period of 35 years from 1852-1887 an incidence of less than 1% was found in Switzerland, Roumania, France and at Erlangen and Kiel in Germany; of 1-2% in Scotland and Russia; of 2-3% at Dresden and Berlin; of 2.5-6% at Leipzig. Where specific figures were cited there is a total of 8,551 necropsy cases examined with only 34 positive cases or an incidence of 0.4%. The early and therefore comparable figures in the U.S.A. at about that time were 5-14.3%, thus even then the highest known incidence in the world. The numbers of clinical cases reported in the U.S.A. as compared with Germany also support this, for since 1915 in the U.S.A. there have been nearly 3,000 cases whilst in Germany, a pork-loving country, only 794 cases have been reported from 1910 to 1936. Statistics in the U.S.A. show that whilst fewer than three people per 100,000 suffer from clinical trichinosis annually, the examination of cadavers proves that about 4,000 per 100,000 are infected annually (McNaught, 1939). In Canada the incidence both in humans and pigs is much lower than in the U.S.A. Kuitunen-Ekbaum, 1941, found seven positives out of 420 autopsies in Toronto; six out of 300 adults and one seven months-old foetus from 120 infants. The first outbreak to be reported in Canada was in Hamilton, Ontario, in 1869 and consisted of five ill and two deaths and a number of cases in Montreal. Recent outbreaks have been reported from the same towns, an outbreak of 23 persons in Hamilton (Deadman & Wilson, 1941) and two outbreaks of seven and 68 cases in Montreal (Gervais, 1938). In Australia no clinical cases have been reported, but in necropsy material in Sydney three positives were obtained, these being old infections of persons who had been resident abroad (Bearup, 1937). No cases of human trichinosis appear to have been reported from the Philippine Islands, Japan or China although the infection has been found in pigs (Manson, 1881, and Koo, 1941) and rats (Tang, 1939) in China and in dogs (Yugawa, 1934) in Manchuria. In the Hawaiian Islands, Alicata, 1938, has

recorded 11 cases over a period of seven years and noted the infection in the rat, mongoose and both wild and domestic pig. In 1942 Lightner & Patterson reported four more human cases. In India a doubtful record of an Englishman dying in Calcutta and trichinae being found in a tumour in his neck occurs as early as 1864, Anon, quoting the Sanitary Commission. In 1907 an outbreak involving 74 Indians with 17 deaths in the Upper Gharwal was reported by Thompson, 1907. The third record of *Trichinella spiralis* in India is of the infection in a cat, the 74th examined in Calcutta by Maplestone and Bhaduri, 1942; the same authors found 100 each of pigs, dogs and rats negative. Records of trichinosis dating from last century exist for Egypt, Syria, Italy, Spain, South America, Russia and Scandinavian countries. Of these the outbreaks in Syria are of particular interest, three having occurred in 1881 (Shrady, 1881, Monsally, 1881 and Wortabet, 1881) and a fourth reported by the latter author in 1883. From that time no further records appear to have been made until 1940 when Saad reported an outbreak affecting 500 persons in Beyrout with a 30% infection of the pigs in that town. Perhaps a high incidence in pigs was one of the age long and wise reasons for the ban on pork in the Jewish race!

Conclusions and Summary.

1. Although outbreaks of trichinosis in Great Britain have been sporadic, the present investigation shows the incidence of *Trichinella spiralis* in man in Southern England to lie between 7.9% and 13.7%, the average incidence being 10.8%. The condition of the larvae, either free or in thin- or thick-walled cysts recovered by the "digest" technique indicated that the infections were of varying ages, from recent to those of many years standing. In six cases there was definite evidence of the presence of both recent and old infections.

Acknowledgements.

I wish to express my sincere thanks to Professor R. T. Leiper for asking me to undertake this work and for his valuable advice and direction. I also wish to thank Dr. B. G. Peters for the statistical analysis of the results and other members of the staff of the Institute of Agricultural Parasitology, St. Albans, for sharing in the testing of the Argentine pork.

Especial thanks is also expressed to the Ministry of Health; to Dr. Jolly, Medical Officer of Health in Wolverhampton; to Mr. Dickinson, Meat Inspector in Wolverhampton and to the following pathologists:

Professor Stewart of Leeds; Dr. Dyke of Wolverhampton; Dr. J. B. Duguid of Cardiff; Dr. T. F. Hewer of Bristol; Dr. R. Williamson of Cambridge and Dr. G. Wilson of Birmingham.

This work was done in the Department of Parasitology, London School of Hygiene and Tropical Medicine and was financed by the Medical Research Council.

References

Alicata, J. E., 1938.—" A study of *Trichinella spiralis* in the Hawaiian Islands." *Publ. Hlth. Rep., Wash.* LIII, 384–393. (W.L. 17302.)

Anon., 1864.—" *Trichina spiralis*." *Lancet.* II, p. 733. (W.L. 11995.)

Armistead, 1879.—" *Trichinosis*." *Lancet.* II, p. 215. (W.L. 11995.)

Badel, A., 1933.—" Zur Frage der Häufigkeit der trichinose beim Menschen." *Wein. klin. Wschr.* XLVI, 302–303. (W.L. 22997.)

Bearup, A. J., 1937.—" A search for *Trichinella spiralis* in cadavers in Australia." *Med. J. Aust. I*, 504–505. (W.L. 13034.)

Beeson, P. B., 1941.—" Factors influencing the prevalence of trichinosis in man." *Proc. R. Soc. Med.* XXXIV, 585–594. (W.L. 16916.)

Brown, T. R., 1897.—" Studies on trichinosis." *Johns Hopk. Hosp. Bull.* VIII, 79–81. (W.L. 10919.)

Cobbold, T. S., 1871.—" Outbreak of trichiniasis in Cumberland." *Brit. med. J.* I, p. 435. (W.L. 3579.)

Deadman, W. J. & Wilson, D. C., 1941.—" Report of outbreak of trichinosis." *Canad. publ. Hlth. J.* XXXII, 513–517. (W.L. 5919.)

Dickinson, W. L., 1871.—" Three cases of trichiniasis after eating home-fed pork." *Brit. med. J.* I, p. 446. (W. L. 3579.)

Fisher, R. A. & Yates, F., 1938.—" Statistical Tables for Biological, Agricultural and Medical Research." Oliver & Boyd, Lond.

Gervais, J. H., 1938.—" Trichinosis in Montreal." *Canad. publ. Hlth. J.* XXIX, 176–179. (W.L. 5919.)

Hall, M. C., 1938.—" Studies on trichinosis. VII. The past and present status of trichinosis in the United States and the indicated control measures." *Publ. Hlth. Rep., Wash.* LIII, 1472–1486. (W.L. 17302.)

Hall, M. C. & Collins, B. J., 1937.—" Studies on trichinosis. I. The incidence of Trichinosis as indicated by post-mortem examination of 3,000 diaphragms." *Publ. Hlth. Rep., Wash.* LII, p. 468. (W.L. 17302.)

Hardy, T. L., 1927.—" A report on two cases of trichinelliasis." *Lancet.* II, p. 1380. (W.L. 11995.)

Harris, J. D., 1909.—" Notes on a sporadic outbreak of trichinosis in Devonshire; with remarks." *Lancet.* I, 1381–1382. (W.L. 11995.)

Howell, N. G., 1942.—" The public health aspect of trichiniasis." *J.R. sanit. Inst.* LXII, 1–5. (W.L. 11498.)

Jolly, R. H. H., 1941.—" Trichiniasis in the West Midlands." *Publ. Hlth., Lond.* LIV, 88–91. (W.L. 17302.)

Kerr, J. B., Jacobs, L. & Cuvillier, E., 1941.—" Studies on trichinosis. XIII. The incidence of human infection with trichinae as indicated by post-mortem examination of 3,000 diaphragms from Washington, D.C., and five eastern seaboard cities." *Publ. Hlth. Rep.* LVI, 836–855. (W.L. 17302.)

Knox, 1845.—" Some observations on the statistics of the vibrio humana. (*Trichina spiralis.*)" *Lond. med. Gaz.* XXXII, p. 805.

Koo, S. Y., 1941.—" Trichinosis among hogs and rats in Fukien." *Nat. med. J. China.* XXVII, 349–350. (W.L. 14838.)

Kuitunen-Ekbaum, E., 1941.—" The incidence of trichinosis in humans in Toronto. Findings in 420 autopsies." *Canad. publ. Hlth. J.* XXXII, 569–573. (W.L. 5919.)

LEIDY, J., 1846.—" On the existence of an entozoon (*Trichina spiralis*) in the superficial part of the extensor muscles of the thigh of the leg." *Proc. Acad. nat. Sci. Philad.* III, 107–108. (W.L. 16590.)

LEWIS, W. L., BOLLER, A. E., HOSKINS, H. P., MERILLAT, L. A. & SMITH, H. R., 1940.—" Trichinosis and non-clinical infections with *Trichinella spiralis*." *J. Amer. med. Ass.* CXIV, 35–39. (W.L. 11006.)

LIGHTNER, G. H. & PATTERSON, W. B., 1942.—" Trichinosis : report of 4 cases." *Hawaii med. J.* I, 302–303.

MCNAUGHT, J., 1939.—" The diagnosis of trichinosis." *Amer. J. trop. Med.* XIX, 181–192. (W.L. 626.)

MANSON, P., 1881.—" *Trichinella spiralis* in Chinese pork." *China Imperial Maritime Customs. Med. Rep., Shanghai.* 21st issue, p. 26. (W.L. 6176.)

MAPLESTONE, P. A. & BHADURI, N. U., 1942.—" A record of *Trichinella spiralis* (Owen, 1835) in India." *Indian med. Gaz.* LXXVII, 193–195. (W.L. 9943.)

MONSALLY, S., 1881.—" Report of the 1st outbreak of trichinosis in Syria." *Med. Rec., N.Y.* XIX, p. 430. (W.L. 13055.)

NOLAN, M. O. & BOZICEVICH, J., 1938.—" Studies on trichinosis. V. The incidence of trichinosis as indicated by post-mortem examinations of 1,000 diaphragms." *Publ. Hlth Rep., Wash.* LIII, 652–673. (W.L. 17302.)

OWEN, R., 1835.—" Description of a microscopic entozoon infesting the muscles of the human body." *Trans. Zool. Soc. Lond.* I, 315–324. (W.L. 21702.)

PAGET, J., 1866.—" On the discovery of *Trichina*." *Lancet.* I, p. 269. (W.L. 11995.)

RANSOM, B. H., 1915.—" Trichinosis." *Rept. 18th Ann. Meet. U.S. Livestock San Assoc.* pp. 1–19.

SAAD, B., 1940.—" La trichinose a propos d'une epidemie observee a Beyrouth." *Pr. med.* XLVIII, 556–557. (W.L. 16550.)

SAWITZ, W., 1938.—" Prevalence of trichinosis in the United States." *Publ. Hlth. Rep., Wash.* LIII, 365–383. (W. L. 17302.)

SHRADY, 1881.—" Trichinosis in Syria." *Med. Rec., N.Y.* XIX, p. 222. (W.L. 13055.)

SOMEREN, V. D. van, 1937.—" The occurrence of sub-clinical trichinosis in Britain. Results from 200 London necropsies." *Brit. med. J.* II, 1162–1165. (W.L. 3579.)

TANG, C. C., 1939.—" *Trichinella* infection in rats in Fukien." *China med. J.* LV, 537–541. (W.L. 6177.)

THOMPSON, C. G., 1907.—" An outbreak of trichinosis in Garhwal." *Indian med. Gaz.* XLII, 373–374. (W.L. 9943.)

TURNER, W., 1860.—" Observations on the *Trichina spiralis*." *Edinb. med. J.* VI, 209–216. (W.L. 7599.)

WORTABET, J., 1881.—" Eine Trichinen-Epidemie am Jordan. Briefliche Mittheilung an den Herausgeber." *Virchows Arch.* LXXXIII, 553–554. (W.L. 22575.) Abs. in *Lancet.* I, p. 454. (W.L. 11995.)

1883.—" Another epidemic of trichinosis near the sources of the Jordan." *Lancet.* II, p. 183. (W.L. 11995.)

WRIGHT, W. H., 1939.—" Studies on trichinosis. XI. The epidemiology of *Trichinella spiralis* infestation and measures indicated for the control of trichinosis." *Amer. J. publ. Hlth.* XXIX, 119–127. (W.L. 617.)

1941.—" The incidence of human infection with the *Trichina* parasite, *Trichinella spiralis*, as indicated by post-mortem examinations." *Meat for Millions. Rep. N.Y. State Trichinosis Commission. Leg. Doc.* LII, pp. 39–54.

WRIGHT, W. H., KERR, K. B. & JACOBS, L., 1943.—" Studies on trichinosis. XV. Summary of the findings of *Trichinella spiralis* in a random sampling and other samplings of the population of the United States." *Publ. Hlth Rep.* LVIII, 1293–1313. (W.L. 17302.)

YUGAWA, T., 1934.—" Trichina found in dogs in South Manchuria." *J. Orient. Med.* XXI, p. 88. (W.L. 11418.)

ZENKER, F. A., 1860.—" Ueber die Trichinenkrankheit des Menschen." *Virchows Arch.* XVIII, p. 561. (W.L. 22575.)

Dates of Publication of Vols. I—XXI.

JOURNAL OF HELMINTHOLOGY

		Date as printed on Cover	*Actual Date of Publication*
Vol. I,	No. 1	March 1923	26th April 1923
	No. 2	May 1923	6th June 1923
	No. 3	July 1923	15th August 1923
	No. 4	September 1923	10th October 1923
	No. 5	December 1923	2nd January 1924
II,	No. 1	February 1924	17th March 1924
	No. 2	April 1924	8th May 1924
	No. 3	July 1924	9th July 1924
	Nos. 4 5	September/ November 1924	8th November 1924
III,	No. 1	February 1925	24th March 1925
	No. 2	May 1925	23rd May 1925
	Nos. 3 4	August 1925	7th August 1925
	No. 5	December 1925	3rd February 1926
IV,	No. 1	March 1926	28th April 1926
	No. 2	May 1926	19th June 1926
	No. 3	August 1926	18th October 1926
	No. 4	November 1926	31st December 1926
V,	No. 1	March 1927	21st April 1927
	No. 2	June 1927	3rd June 1927
	No. 3	September 1927	9th November 1927
	No. 4	December 1927	24th December 1927
VI,	No. 1	March 1928	4th May 1928
	No. 2	June 1928	14th June 1928
	No. 3	September 1928	11th September 1928
	No. 4	December 1928	31st December 1928
VII,	No. 1	March 1929	27th April 1929
	No. 2	June 1929	25th June 1929
	No. 3	July 1929	1st August 1929
	No. 4	December 1929	31st December 1929
VIII,	No. 1	February 1930	10th April 1930
	No. 2	June 1930	16th July 1930
	No. 3	September 1930	20th September 1930
	No. 4	December 1930	30th December 1930
IX,	No. 1	February 1931	18th April 1931
	No. 2	May 1931	16th June 1931
	No. 3	July 1931	1st August 1931
	No. 4	November 1931	15th December 1931
X,	No. 1	February 1932	11th February 1932
	Nos. 2 3	June 1932	8th June 1932
	No. 4	September 1932	6th October 1932

	Date as Printed on Cover	*Actual Date of Publication*
XI, No. 1	January 1933	31st December 1932
No. 2	March 1933	7th March 1933
No. 3	June 1933	31st May 1933
No. 4	October 1933	27th October 1933
XII, No. 1	January 1934	31st January 1934
No. 2	March 1934	29th March 1934
No. 3	June 1934	11th July 1934
No. 4	December 1934	15th January 1935
XIII, No. 1	February 1935	2nd May 1935
No. 2	May 1935	12th June 1935
No. 3	July 1935	2nd September 1935
No. 4	December 1935	31st December 1935
XIV, No. 1	March 1936	14th May 1936
No. 2	June 1936	24th July 1936
No. 3	September 1936	31st October 1936
No. 4	December 1936	31st December 1936
XV, No. 1	January 1937	2nd April 1937
No. 2	April 1937	4th June 1937
No. 3	July 1937	8th July 1937
No. 4	December 1937	30th December 1937
XVI, No. 1	February 1938	18th March 1938
No. 2	May 1938	23rd July 1938
No. 3	August 1938	15th September 1938
No. 4	December 1938	29th December 1938
XVII, No. 1	January 1939	21st February 1939
No. 2	April 1939	26th May 1939
No. 3	August 1939	25th August 1939
No. 4	December 1939	19th January 1940
XVIII, No. 1	March 1940	24th May 1940
Nos. 2/3	October 1940	23rd December 1940
No. 4	December 1940	5th June 1941
XIX, Nos. 1/2	June 1941	8th August 1941
Nos. 3/4	December 1941	27th February 1942
XX, Nos. 1/2	August 1942	16th February 1943
Nos. 3/4	December 1942	24th July 1943
XXI, No. 1	1943	11th March 1944
Nos. 2/3	1945	20th December 1945
No. 4	1946	26th June 1947

The actual dates of publication of the *Journal of Helminthology* are fixed by the date of receipt of the first copy by the Copyright Receipt Office at the British Museum.

R. T. LEIPER.

Journal of Helminthology, Vol. XXII, No. 2, 1948. pp. 63—72.

Observations on a case of Endogenous Budding in *Cysticercus tenuicollis* Rudolphi.

By Hilary Crusz, B.Sc.
Assistant Lecturer in Zoology, University of Ceylon.
(*From the Department of Parasitology, London School of Hygiene and Tropical Medicine.*)

The formation of daughter bladders by endogenous budding appears to be a very rare condition among cysticerci, the only previous record of it, as far as is known, being that of Leuckart in 1886. But in coenuri and echinococci it is the rule rather than the exception. While examining over 400 bladder-worms of *Cysticercus tenuicollis*, obtained at various times from goats slaughtered at the Colombo Municipal Slaughter House, Ceylon, the writer found one cysticercus which showed internal budding. As this specimen was found to be, in many ways, more interesting than the one Leuckart had examined, a description of it is given, together with the results of a study of serial sections which throw light on the way the daughter bladders have been formed.

Description.

The proliferating cysticercus was found together with many normal ones, attached to the mesentery of its host and enclosed within a membrane formed from mesentery. When this covering was torn off, the cysticercus emerged as a large fluid-filled vesicle, about the size of a hen's egg, and measuring 68 mm. by 40 mm. At the narrow end was an opaque thickening somewhat set off from the rest of the bladder, and this carried the neck and scolex which were invaginated. The scolex bore hooks, the large ones of which measured from 190.5μ to 197μ, and the small ones from 126μ to 136μ. Externally the bladder-worm resembled very closely the other specimens obtained from the same host, which were all normal bladder-worms of *C. tenuicollis*.

On opening up this specimen, however, there were to be seen floating freely in the fluid, eight daughter bladders. One of these (9 mm. by 6 mm.) was provided with a distinct invaginated neck and scolex (Fig. 2A) which bore hooks typical of this species, the large ones measuring from 180μ to 194μ, and small ones from 132.5μ to 136μ. This daughter bladder was in every respect a miniature *C. tenuicollis*. Another daughter bladder, of approximately the same size, had no

invagination, but a pad-like thickening at one end of the bladder-wall. The other six daughter bladders were very much smaller, and three of them were distinctly lobed. (Fig. 2B.)

In addition to these free-floating daughter bladders there were about 11 small transparent bladders attached to the parent bladder, in most cases by means of delicate pedicels. (Fig. 1.) Some of these attached bladders were also constricted in more than one place, and appeared to be lobed. The rest of the wall of the parent bladder was covered internally with a large number of small transparent swellings which histological study has shown to be daughter bladders at early stages of development.

An interesting diagnostic feature of the interior of the parent bladder, apart from the shape and size of the rostellar hooks, was the presence of a single, large neck-invagination, bearing an armed scolex from which there trailed into the cavity of the bladder, a long ribbon-like parenchymatous structure. (Fig. 1.) This was identified as the appendage of the receptaculum enclosing the scolex (Leuckart, 1886). It was also to be seen clearly in the daughter cysticercus mentioned above and in all the other normal bladder-worms of this species. In this particular case, however, the end of this appendage was enlarged and showed a number of vesicles not unlike those that developed from the bladder-wall.

Histological Study.

For purposes of histological study, some of the free daughter bladders as well as representative portions of the parent bladder were fixed in Zenker, cleared in methyl benzoate and embedded in paraffin. Serial sections were cut 5μ-8μ thick and stained in Heidenhain's iron alum Haematoxylin or in Chlorazol Black E, without counterstaining. Both stains gave good results.

Parent bladder: The wall of the parent bladder which varies in thickness between 68μ and 374μ, is lined on its outside by a cuticle of more or less uniform thickness (less than 3.4μ), thrown into folds for the most part. Beneath this cuticle is the parenchyma in which the nuclei of its constituent cells appear as intensely staining vesicular bodies. Cell outlines are difficult to observe even under an oil immersion lens, but in many places where the parenchyma is of a looser texture, the cells appear stellate, with cytoplasmic processes traversing the intercellular spaces. A subcuticular layer as such, with spindle-shaped nuclei, is not present, but the cells appear to be more closely packed

together nearer the cuticle than farther away from it. Distinct muscle-fibres are seen running tangentially in the subcuticular region, in some places immediately beneath the cuticle and in some places below one or two cell-layers, while in the inner layers of the parenchyma they run

Fig. 1.—*Cysticercus tenuicollis* showing endogenous budding. Note also the receptacular appendage leading from the invaginated scolex seen at the top of the figure. (x1.)

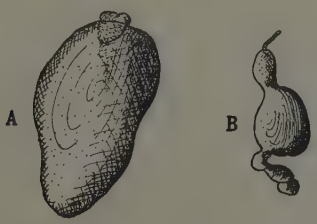


Fig. 2.—*A*. Single daughter cysticercus of *C. tenuicollis*, found within parent bladder and complete with armed scolex and receptacular appendage. *B*. Daughter cyst showing constrictions. (x3.)

obliquely or at right angles to the bladder-wall. The innermost region of the bladder-wall is formed of a layer of fibres which is adjacent to the cavity of the bladder.

Daughter cysts: From the point of view of their position in relation to the parent, it will be convenient to describe three types of daughter bladders, namely, the primitive vesicles originating in the parent bladder-wall itself, the daughter cysts still attached to the parental wall by means of narrow pedicels, and the free daughter cysts,

one of which is a daughter cysticercus. The first two types, as will be seen, are only stages in the development of the free daughter cysts, one of which probably gave rise to the daughter cysticercus.

The wall of the parent cysticercus (Fig. 3A and 4) shows a large number of vesicles of various sizes in its parenchyma. Even the smallest of these vesicles can be distinguished from the very small excretory canals in section, by the fact that it is lined internally by a thin cuticle, distinctly stained both by Haematoxylin and Chlorazol Black. From the fact that there are many cuticular invaginations of the parental wall, some simple and some ramifying, it would appear that these vesicles have been formed by such a process of invagination and have then become separate vesicles. The intermediate stages between the smallest vesicles embedded in parenchyma and the large ones that encroach on the bladder-cavity of the parent, are all represented (Fig. 4). The vesicles then become so distended that they very often reduce the thickness of the parental wall so as to make the latter at times almost imperceptible, and some vesicles lie so close upon one another that they become confluent and form larger vesicles. All these stages only point to the fact that the daughter cysts, still attached to the parent wall by narrow pedicels, have originated by invagination from the parent wall and have subsequently increased in size. These daughter cysts are lined internally by a very distinct cuticle, on the outside of which lies a thin, rudimentary parenchyma. The wall of the daughter cyst is not of uniform thickness. Some portions are relatively thicker than others, and in such regions muscle fibres can be distinguished and the cuticle appears much thicker. In some cases these thickened areas are seen to be invaginated within the daughter cysts and occupy a fair amount of the cavity (Fig. 3A). The cuticle and parenchyma of the invaginated portions appear to go on developing more vigorously. Of course, one cannot say how far these invaginations were due to mechanical causes while handling the material, as some undoubtedly are, but in a few cases the invaginations are particularly striking owing to this difference in the thickness of the walls. Usually there is no more than one thickened area to each daughter cyst.

Finally, there are the daughter cysts which were entirely free (Fig. 3B). These show histological features identical with those of the attached bladders, and there can be no question as to their origin. A very distinct cuticle is to be seen on the inside of their walls and their parenchyma is slightly thicker, and the specially thickened area or areas

appear to be better developed. The histological nature of the single daughter cysticercus found among these free daughter cysts cannot be described, as the entire daughter bladder was used for anatomical study.

Fig. 3.—*A*. Cross-section through portion of parent bladder-wall of proliferating *C. tenuicollis*. *B*. Part of cross-section through one of the free daughter cysts found within parent bladder.

But from the fact that, though small, it was absolutely like its parent in regard to the disposition of the neck, scolex and hooks, and in regard to the parenchymatous appendage of the receptaculum which was found hanging inside its bladder, there can be no doubt that the cuticle in this case is, like the parent cuticle, on the outside.

Discussion.

It is obvious that the bladder-worm described here is of the species *C. tenuicollis*. Externally it appeared to be in every respect a normal *C. tenuicollis*; it was discovered among many other bladder-worms of the same species, infesting the same host, in the same site namely mesentery; it carried rostellar hooks characteristic of the species; one of its daughter bladders too bore very similar hooks and was a normal cysticercus; and both the parent bladder and the daughter cysticercus had typical receptacular appendages.

Although exogenous budding has been known to occur in *C. longicollis* (Bremser 1824, Braun 1896) and has been studied in detail by Bott (1897), nothing definite is known of endogenous budding in cysticerci. Goeze (1782) described a specimen of *C. fasciolaris* which contained a single daughter vesicle in its caudal bladder. He was doubtful, however, of its true nature and origin. Tschudi (1837) reported the occurrence of a number of small bladders of *C. pisiformis* within a larger cyst, but Leuckart (1848) questioned the nature of that "parent cyst", which he took to be the connective tissue sheath of the host. The only genuine instance of endogenous budding appears to be that reported by Leuckart (1886). He described a specimen of *C. tenuicollis* which contained three sterile daughter bladders lying free within the parent bladder.

The present specimen, however, is a clear case of endogenous budding and is unique for the large number of daughter bladders present, in various stages of development, and for the presence among the free daughter bladders, of one daughter cysticercus complete with neck and armed scolex. This is significant because it shows that cysticerci, like coenuri and echinococci, can occasionally reproduce by endogenous budding. In this particular instance, endogenous budding appears to have been neither a means of staving off old age (Clapham, 1940) nor a result of the structural or physiological environment, for if that were so one would have expected to find some similar reaction on the part of the other bladder-worms that were associated with it in the same host animal, which apparently were of the same age. One has to look therefore to some factor or factors intimately connected with the cysticercus itself for an adequate explanation of this phenomenon. The reason may well be in the genes and the whole phenomenon may have an evolutionary significance. What is certain, however, is that endogenous budding is a phenomenon to be reckoned with in all taeniid larvae, in

cysticerci as much as in coenuri and echinococci, and it strengthens the opinion maintained by Southwell and others who classify the adult forms under the one genus *Taenia*, irrespective of the differences that exist between their larval forms.

The histological study of the daughter cysts or vesicles is interesting for the light it throws on their mode of origin. From the many studies made previously, chiefly by Dévé and Coutelen, the daughter hydatids

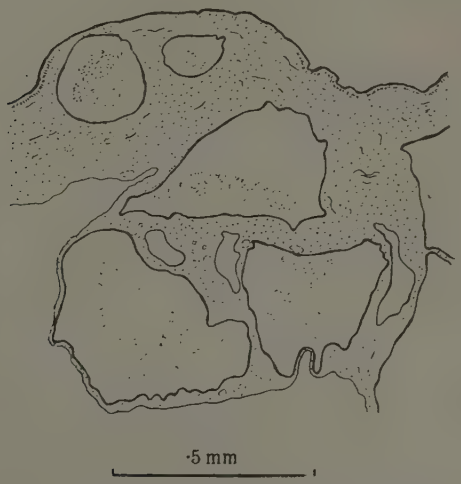


Fig. 4. Cross-section through another portion of bladder-wall of proliferating *C. tenuicollis*, showing developing daughter cysts.

of *Echinococcus granulosus* and the internal daughter coenuri of *Multiceps serialis* appear to be produced by a process of vesiculation of scolices. Daughter cysts produced in this way have their cuticle on the outside and thus have the essential requisite for developmental behaviour similar to that of the parent cysts. If, on the other hand, daughter cysts were produced by simple invagination from the parent wall and subsequent pinching off, then it is certain that the cuticle would, from the beginning, lie within.

Clapham (1940), in particular, has pointed out that endogenous budding could not take place by simple invagination and she found no

evidence for it in *Coenurus glomeratus*. She also stated that if invagination did occur, it would have to be followed either by dissolution of the inner cuticle of the daughter cyst and the secretion of a new one on its outside, or by a turning inside outwards, *i.e.* evagination, of the daughter cyst, resulting in the reversal of layers. She thought, however, that the first method could not be accepted on the principle that cells do not readily change their functions, and that the second method would involve unnecessary complications. Thus the vesiculation of scolices seems to be the only method of daughter cyst formation in echinococci and coenuri.

The present study, however, brings out one fact quite clearly. Daughter cysts *can* be formed by invagination and subsequent pinching off from the parent wall. Such cysts are lined internally by a very distinct cuticle and show thickenings which may well be areas of future scolex formation. But the only daughter cyst to possess a scolex and to be in every respect like the parent cysticercus, except of course in size, had its cuticle on the outside as was to be expected. The absence of scolices throughout the whole extent of the parent wall and in the other daughter cysts, shows that it is highly improbable that the single daughter cysticercus would have developed by the process of vesiculation of a scolex. The presence, however, of a number of free daughter cysts produced by invagination, one of them being of the same size as the daughter cysticercus, points very strongly to a relationship between the daughter cysts with internal cuticle and the daughter cysticercus having its cuticle on the outside. A reversal of layers has most probably taken place, and the mechanism of such reversal may have been by evagination of the wall of a daughter cyst, evidence of which is afforded by sections through some of these cysts. Coutelen (1927) postulated such a process for the brood capsules of *Echinococcus* when he said: "S'étaient d'abord rompues en un point, puis inversées complètement, les scolex étant devenus externes, et enfin reconstituées par soudure des bords de la solution de continuité". The truth of this hypothesis was later confirmed by him histologically: "en effet, les capsules proligères normales sont intérieurement doublées par une mince pellicule cuticulaire sur l'existence de laquelle nous avons insisté dans notre thèse . . . or, cette pellicule cuticulaire ne double plus intérieurement, mais extérieurement les capsules proligères retournées et reconstituées. L'inversion de ces éléments est donc bien réelle; on l'observe très fréquemment dans les cultures".

One cannot help remarking, in conclusion, that the daughter cysts of *C. tenuicollis* described here are, in their mode of origin and their histology, equivalent to brood-capsules of *Echinococcus,* and that, like brood-capsules, they probably have the power to evaginate and reconstitute themselves in such a way that the cuticle comes to lie eventually on the outside. In this way a daughter cyst becomes a daughter cysticercus. Daughter coenuri and daughter hydatids, on the contrary, are formed by vesiculation of scolices and have the cuticle on their outside from the beginning. They are therefore not strictly homologous with the daughter vesicles described here.

Summary.

1. A specimen of *Cysticercus tenuicollis* is described, which contained many daughter cysts in various stages of development. One of these cysts was a well developed daughter cysticercus.

2. The daughter cysts at all stages of development, are lined internally by a distinct cuticle, and have no scolices. The daughter cysticercus, like the parent bladder, has an outer cuticle and a well developed scolex and receptacular appendage.

3. There is evidence for regarding the daughter cysticercus as having been formed from a daughter cyst by a process of evagination of the latter in such a way that the cuticle has come to lie on the outside, rather than by a process of vesiculation of a scolex.

4. The daughter cysts appear to be homologous with the brood-capsules of *Echinococcus*. They are not strictly homologous with daughter hydatids or daughter coenuri.

Acknowledgments.

The writer wishes to express his sincere thanks to Professor J. J. C. Buckley for much help and encouragement; to Professor R. T. Leiper, C.M.G., F.R.S., for help with the literature; and to Professor H. E. Shortt, C.I.E., for his kind help and facilities given at the Institute of Agricultural Parasitology, St. Albans, where the work was carried out in the summer of 1947.

He is also indebted to Mr. S. Saravanamuttu, Superintendent, Municipal Slaughter House, Colombo, for the supply of infested livers and mesenteries of goats. The preliminary study and fixation of material were carried out in the Department of Zoology, University of Ceylon.

References.

Bott, A., 1897.—" Über einen durch Knospung sich vermehrenden Cysticercus aus dem Maulwurf." *Z. wiss. Zool.*, LXIII, 115–140. (*W.L.* 23635.)

Braun, M., 1896.—" Über einen proliferierenden Cysticercus aus dem Ziesel." *Zool. Anz.*, XIX, No. 514, 1–4. (*W.L.* 23833.)

Bremser, J. G., 1824.—Icones Helminthum. *Wien.*

Clapham, P. A., 1940.—" Further studies on *Coenurus glomeratus*." *J. Helminth.*, XVIII, 95–102. (*W.L.* 11224b.)

Coutelen, F., 1927.—" Sur l'évolution vésiculaire in vitro des scolex échinococciques." *Ann. Parasit. hum. comp.*, V, 239–242. (*W.L.* 899a.)
1946.—" Les modes de formation des vésicules filles externes et internes du Cénure sérial." *C.R. Soc. Biol.*, CXL, 258–259. (*W.L.* 6630.)

Dévé, F., 1927.—" La cuticulisation des capsules proligères échinococciques." *Ann. Parasit. hum. comp.*, V, 310–328. (*W.L.* 899a.)
1946.—L'Échinococcose secondaire. *Paris.*

Goeze, J. A. E., 1782.—Versuch einer Naturgeschichte der Eingeweidewürmer thierischer Körper. *Blankenburg.*

Korschelt & Heider, 1936.—Vergleichende Entwicklungsgeschichte der Tiere. Erster Band. *Jena.*

Leuckart, R., 1848.—" Beobachtungen und Reflexionen über die Naturgeschichte der Blasenwürmer." in : *Troschel's Archiv.*
1886.—The Parasites of Man and the diseases which proceed from them. Translated by W. Hoyle. *Edinburgh.*

Southwell, T., 1930.—The Fauna of British India. Cestodes. II. *London.*

Tschudi, A., 1837.—Die Blasenwürmer. *Freiburg.*

Journal of Helminthology, Vol. XXII, No. 2, 1948, pp. 73—76.

On an English case of an intramedullary spinal Coenurus in Man, with some remarks on the identity of *Coenurus* spp. infesting Man.

By Hilary Crusz, B.Sc.

Assistant Lecturer in Zoology, University of Ceylon.
(*From the Department of Parasitology, London School of Hygiene and Tropical Medicine.*)

In February 1947, Mr. D. W. C. Northfield, M.S., F.R.C.S., of the Neuro-surgical Department of the London Hospital, removed by operation a small coenurus from the spinal cord of a 14-year-old girl of Gillingham, who had developed symptoms of paraplegia in December 1946. The girl, who has never lived outside Britain, is presumed to have become infected during a period of evacuation in Wales in 1943-45, where she had had contact with a dog. Professor J. J. C. Buckley, who secured the coenurus through the kind offices of Professor Dorothy Russell, has already made a brief report on it (Buckley, 1947) and has placed at my disposal the original material for detailed study and identification.

Description.

The material, preserved in formal-saline, comprises three fragments, apparently of one coenurus, which had been cut up after removal from the site of infection (see Plate I). The largest fragment is peculiarly branched in a digitate fashion and shows, by its form, that the other two fragments actually belonged to it. It is made up of at least three, more or less elongate, vesicles or lobes, which are confluent at the point of juncture. The largest lobe, measuring about 1 cm. long, bears one group of 4 scolices and another of 2 scolices. A fragment bearing a single cluster of 20 scolices apparently belonged to this lobe. A single pair of scolices is to be found in the second lobe, also 1 cm. long, to which belonged the remaining fragment bearing a group of 3 scolices. The third lobe, which is entire, is 6 mm. long and has no scolices. The original coenurus would therefore have been a distinctly trilobed vesicle containing 31 scolices: the largest lobe containing 26, in groups of 20, 4 and 2; the next containing 5, in groups of 3 and 2; and the smallest being devoid of scolices. The wall of the coenurus is very delicate and transparent, and there is no evidence of any proliferation.

An examination of the scolex-invaginations or buds shows clearly that the coenurus is very immature. They vary in length from about 0.5 mm. to 1.5 mm. There is a great deal of variation in the size of the scolices, even in a single group, and from the various degrees of flexure of the neck region of these scolices it is quite apparent that one is dealing with early developmental stages rather than with mature structures. Examination of the hooks confirms these observations. A scolex bears from 24 to 30 hooks, of which the large ones measure from 119μ to 132.5μ and the small ones from 71.5μ to 85μ. Hooks taken from the smaller scolices are much smaller than those from the larger scolices, and marked differences in shape are also observed, especially in the guard and handle of these structures (Fig. 1). The hooks from small scolices resemble on the whole those of *Coenurus glomeratus*, and some of the hooks from the larger scolices are indistinguishable from immature hooks of *C. serialis* or *C. cerebralis*, while a few resemble mature hooks of these latter species. The blades of the hooks are less variable in shape and size, and are indistinguishable from the hook-blades of any of the three species mentioned. What is perhaps the most convincing evidence of the immaturity of this coenurus is the fact that the majority of the hooks, even some of the largest, are flexible, incompletely sclerotized structures.

From all this evidence of the immaturity of this coenurus, and from a comparison of this material with plenty of other material of various species obtained from man, goat, sheep and various rodents, it is concluded that the species is *Coenurus cerebralis* (*Multiceps multiceps*). This would be the second case on record of the occurrence of human coenurosis in Great Britain. In this instance the patient had never been abroad and had thus acquired the infection in this country.

Identity of *Coenurus* spp. found in Man.

Coenurosis in man has hitherto been reported about a dozen times, and three species have been implicated, namely *C. cerebralis*, *C. serialis* and *C. glomeratus*. *C. cerebralis* has been recorded from Africa, France and England and has always been found to infest the brain. *C. serialis* has been recorded from France and the U.S.A., the site of infection being chiefly the intermuscular connective tissues, although one coenurus obtained from the brain of a Californian boy has been tentatively diagnosed as *C. serialis* and reported by Faust in 1943. *C. glomeratus* has so far been reported only from Africa.

A case particularly interesting on account of the difficulty experienced in identifying the coenurus on the basis of size and shape of hooks, is that reported by Taramelli and Dubois (1931), who were unwilling to name the specimen which they had obtained from the right fore-arm of a native woman of the Belgian Congo. Baylis (1932), who examined a portion of the same cyst, came to the conclusion that it

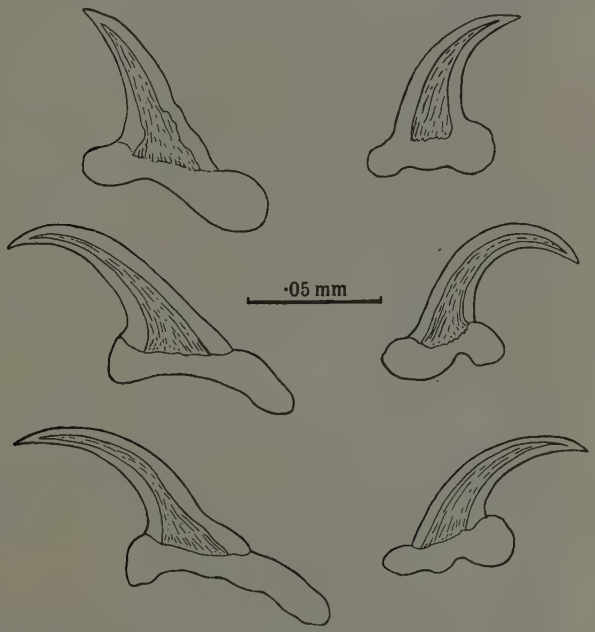


Fig. I. Large and small hooks from three different scolices of immature *Coenurus cerebralis* from spinal cord of Man.

was impossible to identify the species definitely with either *C. cerebralis* or *C. serialis*. The coenurus described here offers a similar, if not more significant, difficulty, in that it shows many scolices with hooks resembling those of *C. glomeratus*, although some scolices bear hooks not different from those of *C. serialis* or *C. cerebralis*.

Nagaty and Ezzat (1946) have drawn attention to the presence of a ventral swelling in the handle of the small hook of *C. serialis*, and it was thought that this might offer a clue to the identification of the present specimen. Such a projection was clearly seen in some of the

small hooks, but by no means in all. Examination of more larval and adult material of *T. serialis* from rabbits, hares and dogs, proved that this cannot be regarded as a feature of any diagnostic value.

Various characters other than shape and size of hooks have from time to time been invoked as valuable aids to the differentiation of these three species. It has been said that in *C. cerebralis* the scolices develop in clusters or groups, whereas in *C. serialis* they are irregularly scattered; that *C. cerebralis* is a parasite of the central nervous system and does not show branching or proliferation, whereas *C. serialis* is a parasite of the intermuscular connective tissue and often shows proliferation by endogenous or exogenous budding. The present specimen, however, shows scolices in distinct clusters, and also a peculiar digitate type of branching, unusual in *C. cerebralis* and somewhat like the dendritic type of proliferation observed by Schwartz (1924) in the bladder-worm of *T. twitchelli*.

In view of all these difficulties it is important that due consideration be given to the possibility, stressed by Southwell (1930), Baylis (1932) and Clapham (1942) and by certain German and Italian workers before them, that helminthologists have hitherto been dealing with a single species of *Coenurus* infesting man, and that they have erroneously regarded different developmental stages of this species, as three distinct species. Clapham (1942) has already shown, statistically and otherwise, that the case for a single species, namely *C. cerebralis* (*Multiceps multiceps*) of which *C. serialis* would be at most only a variety or physiological "strain", and *C. glomeratus* a very early stage in its development, is in best accord with experience. The coenurus described here offers further evidence in this direction.

References.

Baylis, H. A., 1932.—"On a *Coenurus* from man." *Trans. Roy. Soc. Trop. Med. & Hyg.*, XXV, 275–280. (*W.L.* 21671.)

Buckley, J. J. C., 1947.—"*Coenurus* from human spinal cord." *Trans. Roy. Soc. Trop. Med. & Hyg.*, XLI, 7. (*W.L.* 21671.)

Clapham, P. A., 1941.—"An English case of *Coenurus cerebralis* in the human brain." *J. Helminth.*, XIX, 84–86. (*W.L.* 11224b.)

1942.—"On identifying *Multiceps* spp. by measurement of the large hook." *Ibid.*, XX, 31–40.

Craig, C. F. & Faust, E. C., 1943.—Clinical Parasitology. 3rd edition. *London.*

Nagaty, H. F. & Ezzat, M. A. E., 1946.—"On the identity of *Multiceps multiceps* (Leske, 1780), *M. gaigeri* Hall, 1916, and *M. serialis* (Gervais, 1845), with a review of these and similar forms in man and animals." *Proc. Helm. Soc. Wash.*, XIII, 33–44.

Schwartz, B., 1924.—"A new proliferating larval tapeworm from a porcupine." *Proc. U.S. Nat. Mus.*, LXVI, Art. 24, 1–4. (*W.L.* 16944.)

Southwell, T., 1930.—The Fauna of British India. Cestodes. II. *London.*

Taramelli, N. & Dubois, A., 1931.—"Un cas de Coenurose chez l'Homme." *Ann. Soc. Belge. Méd. Trop.*, XI, 151–154. (*W.L.* 926.)

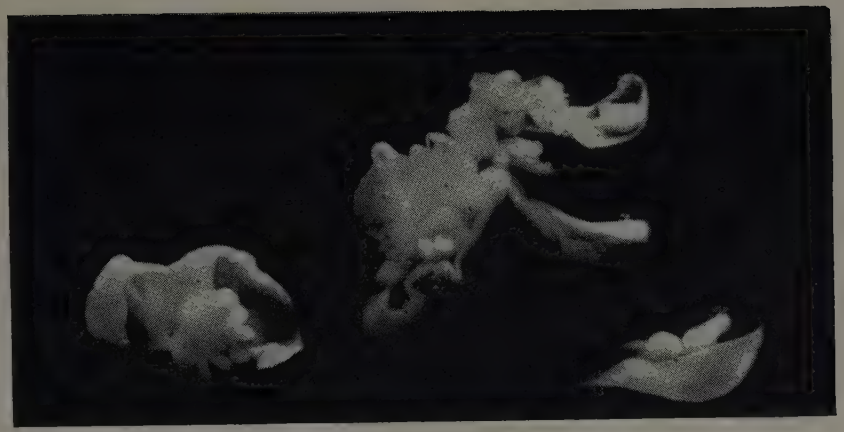


PLATE I

Photograph of *Coenurus cerebralis* from spinal cord. (x4). *Left* : fragment with cluster of 20 scolices. *Centre* : coenurus showing digitate form. *Right* : fragment with group of three scolices.

Journal of Helminthology, Vol. XXII, No. 2, 1948, pp. 77—92.

Studies on the Helminth Parasites of Birds in Hyderabad State. Nematoda I.

By Satya Narayan Singh, M.Sc.,

Reader-in-charge in Zoology, Osmania University, Hyderabad Deccan.
(From the Department of Parasitology, London School of Hygiene and Tropical Medicine.)

The round worm parasites of birds are a very interesting group of nematodes, worthy of a detailed and comprehensive study. Various helminthologists working on nematode parasites of vertebrates in India have reported casually on bird nematodes from Hyderabad State. As the avian fauna of this State, besides being rich and varied has considerable economic value, it was considered that an investigation of their parasites would prove to be both valuable and interesting. With this object in mind the writer, in the year 1940, began collecting bird helminths at Hyderabad, and thus acquired the material which forms the basis of the present study.

Material and Technique.

The birds which were sacrificed for this purpose were from Hyderabad, and either they were procured from the local bird-market or they were shot in the suburbs of the city. Before opening the body cavity, the eyes of the birds were carefully examined and in few instances some species of *Oxyspirura* were recovered. After this the birds were dissected and subcutaneous connective tissue and various body cavities were searched for the presence of Filariids. Next the alimentary canal was removed and different regions of it were examined separately in a Petri-dish under a binocular microscope. It was observed that the site of heaviest infection was the gizzard in which spirurid worms, such as *Acuaria* and *Schistorophus* species were found lying under the corneous lining. A common parasite of the proventriculus was *Tetrameres*, the females of which could be squeezed out easily from the glands of this organ. The males occurred freely in the lumen but were very rare. Species of *Echinuria, Synhimantus, Dispharynx,* and *Cheilospirura* were collected from the buccal cavity and the oesophagus.

In the caeca various species of *Subulura* and *Heterakis* were found frequently. Filariids were harboured in the heart but they were very rare. During the collection work which extended over a period of two years, it was observed that on the whole the incidence and intensity of

infections were greater in the dry season than during the rains, probably due to the external factors governing the development of larval nematodes.

Live specimens of worms were examined under the microscope in one per cent. normal saline in order that delicate structures such as the cephalic papillae of the head and the caudal papillae in the male, which are sometimes difficult to observe in fixed and preserved material, could be studied in detail. The worm was mounted on the slide in saline and then it was gently heated on a water bath until its movements ceased completely. For the study of preserved material the worms were fixed in 70% alcohol or 5% formalin for 24 hours and then stored in 70% alcohol with 5% of glycerine. Medium sized and small worms were mounted in glycerine or glycerine-jelly, but for large worms with thick cuticle, lactophenol gave good results. Dehydration and clearing was done by allowing the glycerine-alcohol mixture to evaporate gradually at room temperature until the worms were left in almost pure glycerine. Glycerine-jelly was a useful mounting medium for obtaining an end-on view of the head, as it facilitates orientation while in melted condition and also has the advantage of setting solid with the object in the desired position.

NEMATODA.

Desmidocercidae Cram, 1927.

Desmidocercella Yorke and Maplestone, 1926.

Syn., *Desmidocerca* Skrjabin of Seurat, 1920.

Desmidocercella leiperi n.sp.

In October 1946, several worms belonging to this species were collected from the air-sacs of an Indian Pond-heron, *Ardeola grayii*. The material consists of young and fully grown individuals of both sexes. The worms, after being examined in live condition, were fixed, some in 70% alcohol and others in 5% formalin. It was found that while they coiled in alcohol, they remained extended in formalin.

These are small worms with no marked difference in size between the sexes. In the female, as well as in the male, the posterior end of the body is flexed ventrally. The maximum width is reached in the post-oesophageal region, whilst towards each extremity the body tapers slightly to terminate in a bluntly pointed head or tail end. The cuticle, which is thin and devoid of transverse striations, is marked with fine longitudinal lines. The lateral fields are quite prominent and the alae,

faintly developed in the male, are lacking in the female. The cervical papillae could not be observed even after prolonged search. The nerve ring encircles the muscular portion of the oesophagus towards its posterior end and slightly posterior to this the excretory pore opens on the mid-ventral line of the body. The excretory system is connected to the excretory pore by means of a reservoir.

The opening of the mouth is bounded by a cuticular band or ring which presents a trilobed appearance in a lateral view (Fig. 4). The cephalic papillae are best seen in an end-on view. They comprise three pairs situated towards the apex of the head, and two pairs situated towards its base. The "amphids" are located laterally and appear circular in outline. The mouth opens into a cylindrical buccal capsule which is provided anteriorly with a pair of laterally situated onchia (Fig. 3). In lateral view the capsule assumes a conical shape due to the fact that the onchia appear as inwardly projecting anterior prolongations of its walls. The oesophagus is cylindrical and is divided into two portions, a short anterior muscular portion, and a long posterior glandular portion. The oesophago-intestinal valves are as in *Desmidocercella numidica* (Seurat, 1920). The intestine runs in a straight course towards the posterior end and in the female it is connected by a short rectum to the anus.

Female. The females vary in length from 3.175 to 4.53 mm. with a maximum width of 127-185μ. The buccal capsule measures 23μ. The excretory pore is 176-180μ from the head end. The muscular and glandular portions of the oesophagus are 104-145μ and 384-427μ long respectively; they together constitute 1/6 to 1/7 of the body length. The tail is 42-58μ long and forms roughly 1/75 to 1/78 of the body length. The cuticular knobs are present on the tail, but are not so prominent as in the female.

The vulva is flush with the body surface and is post-oesophageal in position. It is situated at a distance of 540-770μ from the anterior end. The vagina is a short tube with thick cuticular lining. It leads into a posteriorly directed ovejector. The latter is composed of two portions, a long and thick walled muscular pars ejectrix, and a relatively short bulb-like pars haustrix. Running parallel from the vagina are two posteriorly directed uteri. One of these turns forwards at the middle of the body and is connected anteriorly to a short spermatheca having a slightly expanded lumen. The oviduct which follows is a narrow tube; it forms a loop and turning backwards is continuous with the filiform

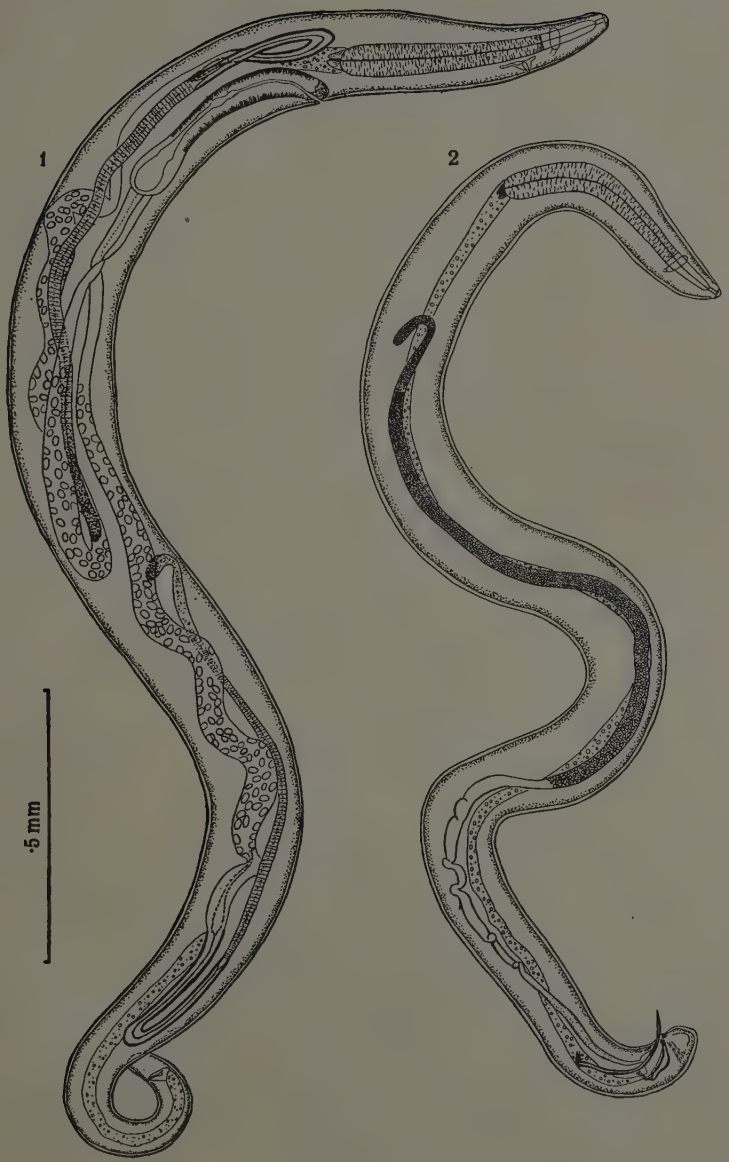
ovary. The latter extends down to the middle of the body. The other uterus runs straight backwards to end in a spermatheca of the same length as the anterior one. It leads into the oviduct which is reflexed and continues into the anteriorly directed ovary. The tips of the two ovaries are just opposite to each other. From the preceding account of the female reproductive system it is clear that there are two flexures in the anterior genital tube, whereas there is only one in the posterior one. In the former the flexure occurs at about the middle of the uterus and again in the oviduct, whilst in the latter there is only a single flexure, formed by the oviduct. The eggs in the uteri measure 14 to 26μ by 10 to 12μ. They are unsegmented. No eggs were found in the ovejector.

Male. The male is 2.78 to 4 mm. long by 113 to 171μ wide. The buccal capsule measures 23μ in length. The excretory pore is situated at a distance of 173μ from the anterior end. The muscular and the glandular portions of the oesophagus are 91-137μ and 378-427μ long respectively. They together form 1/6 to 1/7 of the body length. The cloacal aperture is 40 to 60μ from the tip of the tail, which forms roughly 1/69 of the body length.

There is a single testis occupying a considerable length of the body. Its anterior end extends into the anterior third of the body and is reflexed. Posteriorly it is followed by a slightly convoluted vas deferens. The seminal vesicle, which is not well differentiated, opens by means of a short ejaculatory duct into the cloaca. The tail is short and bluntly rounded, there being no caudal alae. The sessile caudal papillae are difficult to observe, and require the oil-immersion objective for their examination. There is a single pair immediately posterior to the cloacal aperture and two pairs anterior to it. In addition, the tail carries a pair of knob-like lateral cuticular swellings, which are prominent only in lateral view. The spicules, which are dissimilar in shape and unequal in length, measure 225-241μ and 106-116μ. They are curved ventrally and each is provided with a handle-like anterior end and is pointed towards its tip. The large spicule is tubular, whereas the small one is much broader and has an open cavity ventrally.

Desmidocercella leiperi n.sp.

Figs. 1 & 2. Female and male respectively in side view, showing general shape and structure.


1
2
·5 mm

Discussion.

The genus *Desmidocerca* was created by Skrjabin, in 1916, for a very small nematode which he found in the air-sacs of *Ardea cineria,* and which he named *Desmidocerca aerophila*. Later, in 1920, Seurat described a second species *Desmidocerca numidica from* "Héron cendré". Yorke and Maplestone (1926) recognised the fact that caudal papillae in the male while absent in the type species *Desmidocerca aerophila* are present in *Desmidocerca numidica*. They accordingly formed a new genus, *Desmidocercella,* to accommodate the latter species. Since then a second species, *Desmidocercella incognita* was added by Ssolonitzin (1932). The species described herein forms a third species of the genus. In the light of the knowledge of the various species of the two genera it may be noted that the main difference between them is concerned with the relation of the oesophagus length to the body length. In *Desmidocerca* the oesophagus is very long, about 2/3 the length of the worm, whereas in *Desmidocercella* it is relatively very short, about 1/6 to 1/9 of the body length. As regards the caudal papillae it may be pointed out that as they are very small they are likely to be missed in a lateral view of such a small worm as *Desmidocerca aerophila.*

The important measurements which serve to differentiate the three species of *Desmidocercella* are given in the following table.

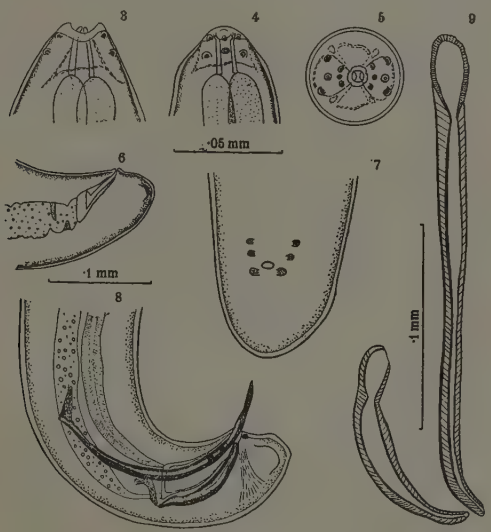
Measurements (in millimetres) of species of *Desmidocercella.*

Species	*D. numidica*	*D. incognita*	*D. leiperi*
Length (male)	5.3	4.25	2.78-4
Length (female)	6.8	4.4-5	3.175-4.53
Proportion of oesophagus to body length	1/8 to 1/9	1/9	1/6 to 1/7
Length of spicules	.48 & .155	?	.225-.241 & .106-.116
Spicule ratio	2 : 5	1 : 3	1 : 2
Host	"Héron cendré"	*Phalacrocorax carbo* L.	*Ardeola grayii*

Besides differing from known species in important measurements the present worm possesses two important features:

1. The vulva is posterior to the junction of the oesophagus and the intestine, whereas in *D. numidica* it is anterior to the junction, and in *D. incognita* it is in the posterior part of the body.

2. In the male of this species there are two pairs of pre-anal and one pair of post-anal papillae, whereas in *D. numidica* there is only a single pair, post-anal in position. It differs from *D. incognita* in possessing only one pair of post-anal papillae. Moreover, the pre-anal papillae in this worm are much closer together than in *D. incognita*.



Desmidocercella leiperi n.sp.

Fig. 3.—Head end, ventral view. Fig. 4.—Head end, lateral view. Fig. 5.—End-on view of the head. Fig. 6.—Posterior end, female, lateral view. Fig. 7.—Posterior end, male, ventral view. Fig. 8.—Posterior end, male, lateral view. Fig. 9.—Spicules, ventral view.

(Scale .05 mm. applies to Figs. 3, 4 and 5 ; the smaller scale .1 mm. applies to Figs. 6 and 8 ; the larger scale .1 mm. applies to Figs. 7 and 9.)

In view of the differences exhibited by the worm, it is considered to be a new species and the writer proposes to name it *Desmidocercella leiperi* after Professor R. T. Leiper, F.R.S.

Host : Ardeola grayii.

Habitat : Air-sacs.

Locality: Hyderabad Deccan (The Nizam's Dominions).

Type specimens will be deposited in the Parasitology Department of the London School of Hygiene & Tropical Medicine.

Thelaziidae Railliet, 1916.

Oxyspirura Drasche in Stossich, 1897.

Oxyspirura mansoni (Cobbold, 1879) Ransom, 1904.

This worm was recovered twice from the orbital cavity of the Red Spur-Fowl, *Galloperdix spadicea* (Ver : Jungli Murghi), a single male specimen on one occasion and one female and one male on another occasion. The worms were found crawling about on the eye-ball just under the nictitating membrane.

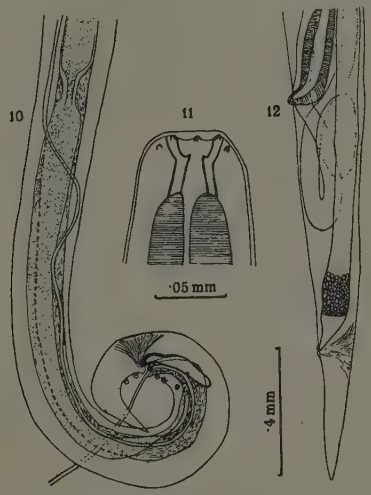
The body is attenuated towards the extremities in both sexes. The male is slender and smaller in size, whereas the female is stouter and its length is about one and a half times that of the male.

The cuticle is thin and devoid of transverse striations, and is marked with longitudinal lines, which are very prominent in the middle region of the body. The head bears two pairs of sub-median and one pair of lateral papillae (Amphids). The excretory pore is posterior to the level of the nerve ring and is situated at a distance of 390μ from the anterior end in the male. The nerve ring encircles the anterior portion of the oesophagus and is 225μ from the anterior end in the male, and 275μ in the female.

The mouth which is surrounded by a cuticular ring, leads into a buccal capsule. The latter is divided into two portions—a short expanded anterior and a relatively longer posterior portion; it measures 35μ in length in the male and 42μ in the female. The oesophagus is cylindrical in shape, and its total length is 1.365 mm. in the male and 1.47 mm. in the female; it forms roughly 1/9 to 1/11 of the body length. It consists of a short anterior muscular and a long glandular posterior portion. The two constituent portions measure .28 and 1.085 mm. in the male and .31 and 1.16 mm. in the female. The intestine is a narrow tube and in the female it communicates with the exterior through a narrow rectum.

Female : The female is 17.5 mm. long with a maximum diameter of 390μ. From some distance in front of the vulva the body begins to narrow and ends in a conical and pointed tail. The vulva is 760μ in advance of the anal aperture. The vagina is directed anteriorly and the uteri are parallel. The exact relation between the ovaries, oviducts and the uteri could not be made out due to the fact that the specimen under observation was a gravid female, crowded with eggs in the genital ducts. The eggs are oval, 35-42μ long and 22-27μ broad.

Male : The male attains a length of 12.2 mm. with a maximum thickness of 248μ. The posterior extremity is strongly flexed ventrally and forms a sharply pointed tail. The caudal alae are absent. The distance from the cloacal aperture to the tip of the tail is 240 to 300μ. There are seven pairs of caudal papillae situated on the ventral surface of the tail; three pairs are pre-anal in position, two pairs post-anal, and one ad-anal. In addition to these, there is a rather indistinct pair of papillae near the tip of the tail. There is a single testis occupying a



Oxyspirura mansoni

Fig. 10.—Posterior end, male, lateral view. Fig. 11.—Head end, lateral view. Fig. 12.—Posterior end, female, lateral view.

considerable length of the body. It communicates by means of a vas deferens, of the same diameter as itself, with a slightly expanded tube, the seminal vesicle. The ductus ejaculatorius is fairly long, and opens at one end into the seminal vesicle and at the other into the cloaca. There are two spicules which are unequal in length and dissimilar in shape. The right spicule, which is long and thin, measures 4 to 4.2 mm. in length; it is slightly expanded at its anterior end, whilst its distal end terminates in a blunt point. The left spicule, which is 225 to 240μ in length, is trough-like, with a distinct broader posterior end.

Hitherto this worm has been reported from the orbital cavity of the fowl (*Gallus gallus*), the turkey (*Meleagris gallopavo*), and in the

peafowl (*Pavo cristatus*) in various parts of the world. The writer here records it from a new host—the Red Spur-fowl, *Galloperdix spadicea*.

Host : The Red Spur-fowl, *Galloperdix spadicea*.

Habitat : Orbital cavity.

Locality: Hyderabad Deccan (The Nizam's Dominions).

Oxyspirura (*Cramispirura*) *popowi* Skrjabin, 1929.

One male and three females of this species were obtained from the orbital cavity of "Dhayal" (Magpie-Robin), *Copsychus saularis*. In both sexes the body is attenuated towards the extremities, more so in the region of the tail. The cuticle is thin and without transverse striations. There are present, however, delicate longitudinal folds along the whole length of the body. The head is broadly rounded and is not set off from the body. In common with other species of the genus *Oxyspirura* the head bears two pairs of submedian and one pair of lateral papillae. The "amphids" are placed midway between the posterior pair of sub-median papillae. The buccal capsule is cylindrical, undivided and leads into an oesophagus formed of two portions. The nerve ring crosses the anterior portion of the oesophagus near its junction with the glandular portion. The excretory pore opens further down on the mid-ventral surface of the body.

Female : The female is slightly larger than the male and measures 8.6 to 8.9 mm. in length and 383μ in maximum width. The distance from anterior end to the nerve-ring is 149μ, to the excretory pore 250μ. The two portions of the oesophagus measure 1.54 and .457 mm. respectively, the total length thus being .611 mm.

Unlike the male, the posterior extremity of the female begins to narrow from a considerable distance in front of the anus. The anal aperture is 257μ from the tip of the tail and the vulva is 189μ anterior to it. The muscular sphincter round the vulva is well developed. The vagina is directed anteriorly and the paired uteri run parallel throughout their course. The ovaries are filiform and extend into the neighbourhood of the vagina. The eggs in the uteri were embryonated and measured 25 to 39μ by 12 to 20μ.

Male : The male, which is somewhat smaller than the female measured 5.75 mm. in length and 219μ in transverse diameter. The buccal capsule is 28μ broad. The total length of the oesophagus is 611μ and its two portions measured 183μ and 428μ respectively. The posterior extremity is coiled in a single turn. The cloacal opening is

situated on a projecting portion of the ventral body wall. Its distance from the tip of the tail is 148μ. The caudal alae are lacking. The papillae are sessile and each has a spike-like process at its top. There are altogether seven pairs, three of which are pre-anal and four post-anal in position. Of the post-anal ones, the most posteriorly placed is located laterally. In the present worm the first pair of post-anal papillae, which Skrjabin (1931) describes as being ad-anal, lies immediately posterior to the level of the cloaca, though somewhat displaced laterally.

Oxyspirura popowi

Fig. 13.—Head end, lateral view. Fig. 14.—Posterior end, female, lateral view. Fig. 15.—Anterior end, female, lateral view. Fig. 16.—Posterior end, male, lateral view. Fig. 17.—Spicules, lateral view.

The spicules are small and sub-equal, measuring 157μ and 120μ respectively. In the large spicule the maximum diameter is reached in its anterior half; it is boat shaped with a broad proximal extremity and a pointed distal extremity. The short spicule possesses a relatively broader head portion and is pointed towards the tip. It is provided with a transparent wing-like lateral expansion marked with fine transverse striae. The vas deferens is of the same diameter as the testis and is crowded with spermatozoa. The ejaculatory duct is fairly long with a distinct bulb-like enlargement near its junction with the seminal vesicle.

Host : Copsychus saularis.

Habitat : Orbital cavity.

Locality: Hyderabad Deccan (The Nizam's Dominions).

Oxyspirura indica n.sp.

Three specimens of *Kittacincla malabarica* (Vernacular : "Shama") were examined for the presence of eye worms and one was found infected with *Oxyspirura*. In all three specimens were collected, one male and two females.

The worms, when fresh, appear yellow in colour, which makes them easy to detect amongst the nerves and recti muscles of the eye. The body is attenuated towards the extremities in both sexes. The male is slender, whereas the female is stout and slightly larger.

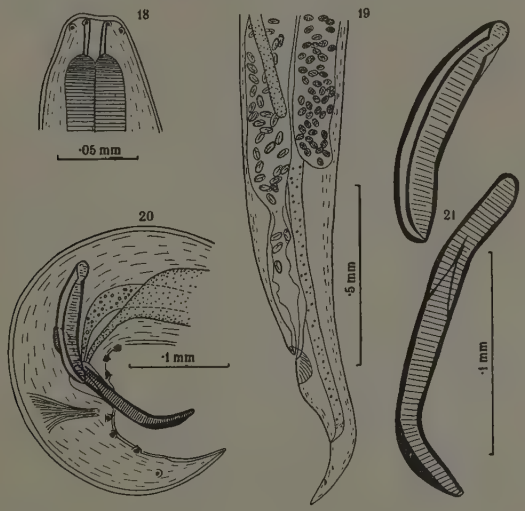
The cuticle is thin and lacking transverse striations, but is marked with fine longitudinal striae. The head is broad with rounded sides. The cephalic papillae comprise two pairs of sub-median and one pair of lateral papillae. The undivided and cylindrical buccal capsule is 22μ long and is followed by an oesophagus which consists of a small muscular and a long glandular portion measuring 149μ, 169μ and 553μ respectively. The total length of the oesophagus constitutes 1/10 to 1/12 of the body length. The nerve ring encircles the muscular portion of the oesophagus and is situated at a distance of 149μ to 154μ from the anterior end. The excretory pore opens on the mid-ventral line 308μ from the head end.

Female: The female measures 7.45 to 8.84 mm. in length and 387μ to 412μ in maximum width. The posterior end of the body narrows gradually to end in a pointed tail which is slightly curved ventrally. The anus is 185μ from the tip of the tail and 256μ from the vulva. The sphincter muscle round the vulval opening is well developed. The vagina is directed anteriorly. The two uteri run side by side towards the anterior end and on approaching the junction of the oesophagus and the intestine they are reflexed and run backwards until they reach the vagina. Here they form a second flexure and run once again anteriorly to be connected to the oviducts which are followed by the posteriorly directed ovaries. The latter extend down to the point of the origin of the uteri. The tip of one ovary is slightly in advance of the other. The worms examined were gravid females with their genital ducts fully packed with eggs in various stages of development. The embryonated eggs in the vagina and the uteri measured 30 to 42μ long by 20 to 23μ wide.

Male : The male attains a length of 5.83 mm. with a maximum diameter of 258μ. Its posterior extremity is strongly flexed ventrally and describes a circle with the result that the tip of the tail is

overlapped by the body. The cloacal aperture is 144μ from the tip of the tail.

The testis is filiform and runs straight until it reaches the vicinity of the oesophagus where it is coiled in two turns and terminates in a short posteriorly directed tip. It is followed by the vas deferens which leads into the seminal vesicle. The latter opens by means of a distinct ejaculatory duct into the cloaca. The tail is short and pointed and lacks caudal alae. There are three pairs of pre-anal and four pairs of post-anal papillae. Of the post-anals the last pair is situated laterally. The



Oxyspirura indica n.sp.

Fig. 18.—Anterior end, lateral view. Fig. 19.—Posterior end, female, lateral view. Fig. 20.—Posterior end, male, lateral view. Fig. 21.—Spicules, lateral view.

spicules are 127 and 182μ respectively. The smaller one is shoe-shaped and is broader than the large spicule which is rather narrow and tubular.

This species is closely related to *O.* (*Cramispirura*) *baskakowi* Skrjabin, 1929 and *O.* (*Cramispirura*) *popowi* Skrjabin, 1929. However, it differs from both in the number and arrangement of the caudal papillae, or in the size and shape of the spicules. *O. baskakowi* possesses four pairs of pre-anal and two pairs of post-anal papillae, whereas *O. indica* has three pairs of pre-anal and four pairs of post-anal papillae. The spicules of the new species differ considerably from those

of *O. popowi.* Its large spicule is relatively longer and of uniform thickness, but in *O. popowi* it is somewhat smaller and reaches its maximum thickness in the middle of its anterior half. The short spicule of *O. indica* though it does not differ much in size from the corresponding spicule of *O. popowi,* has a distinct alate expansion which is lacking in the latter. Ad-anal papillae are lacking in the present worm, whereas they are present in *O. popowi.*

Host : Kittacincla malabarica.

Habitat : Orbital cavity.

Locality: Hyderabad Deccan (The Nizam's Dominions).

Type specimens will be deposited in the Parasitology Department of the London School of Hygiene and Tropical Medicine.

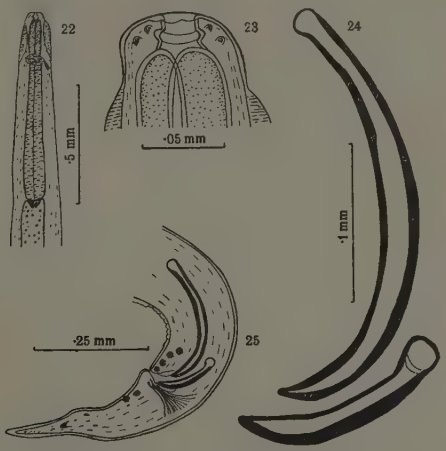
Oxyspirura buccosulcata n.sp.

A single male specimen was obtained from the orbital cavity of the Brahminy or Black-headed Myna (*Temenuchus pagodarum*). The worm was found crawling about over the eye-ball, just underneath the nictitating membrane.

This is a rather small worm which in live condition appears yellow in colour. The body is of uniform thickness and while narrowed towards the anterior end, it forms a sharply pointed tail posteriorly. The solitary male measured 8.94 mm. in length and 265μ in width. The cuticle, which is thin and without transverse striations, bears longitudinal ridges running along the whole length of the body. The head is truncated with rounded sides and its cuticle forms a pair of wing-like membranous expansions, situated laterally. They are transparent and marked with delicate transverse striae. The excretory pore is located on the mid-ventral line of the body in the region posterior to the nerve ring. The latter crosses the muscular portion of the oesophagus near its junction with the glandular portion.

The mouth is surrounded by a cuticular rim. Of the cephalic papillae only two pairs of sub-medians could be seen. The lateral papillae, however, could not be distinguished. The mouth leads into a short buccal capsule measuring 22μ long. It is shaped as shown in Figure 23. At its posterior end it widens out a little. One notable feature of the capsule is that at about the middle of its length there is an internal circular groove, which appears like a ridge on its external surface. The anterior muscular portion of the oesophagus measures 123μ and the posterior glandular portion 564μ in length. The two portions together form 1/12 of the body length.

The tail is flexed ventrally and presents a hook-like appearance at the posterior end of the body. It is sharply pointed and without caudal alae. The caudal papillae are sessile and disposed symmetrically. There are seven pairs altogether, out of which four are pre-anal and three post-anal in position. Of the pre-anal ones, the most anteriorly located are lateral in position. The rest of the papillae form a single series on each side. In addition, an isolated pair is situated laterally near the tip of the tail. The spicules are unequal and measure 300μ and 140μ respectively. The anterior end of the large spicule is not distinctly set off, whereas the short one has a distinct swollen end. The tips of both spicules are pointed.



Oxyspirura buccosulcata n.sp.
Fig. 22.—Anterior end, lateral view. **Fig. 23.**—Head, lateral view. **Fig. 24.**—Spicules, lateral view. **Fig. 25.**—Posterior end, male, lateral view.

The species bears resemblance to *O.* (*Oxyspirura*) *brevisubulata* (Molin, 1860) Stossich, 1897, in having two cephalic expansions of the cuticle and also in the ratio of spicule length. Unfortunately the spicules are not figured and their measurements too are not given. The short spicule is mentioned as being thick. In the present worm however both spicules are approximately of the same thickness. The number of post-anal papillae is differing in these two worms. While there are (?) eight pairs in *O. brevisubulata,* there are only three pairs in *O. buccosulcata.* The new species can be further distinguished by its smaller size and the characteristic shape of the buccal capsule.

Host : Temenuchus pagodarum.

Habitat : Under the nictitating membrane.

Locality: Hyderabad Deccan (The Nizam's Dominions).

Type specimen will be deposited in the Parasitology Department of the London School of Hygiene and Tropical Medicine.

Summary.

Desmidocercella leiperi n.sp. from *Ardeola grayii* is described in detail and compared with other related species.

Two species of *Oxyspirura* are recorded from new hosts. *Oxyspirura mansoni* from *Galloperdix spadicea* and *O.* (*Cramispirura*) *popowi* from *Copsychus saularis.*

Two new species of *Oxyspirura* are described, *O.* (*Cramispirura*) *indica* n.sp. from *Kittacincla malabarica* and *O. buccosulcata* n.sp. from *Temenuchus pagodarum.*

Acknowledgments.

The entire work was carried out under the guidance of Professor J. J. C. Buckley to whom the writer wishes to tender his gratitude. Thanks are also due to Professor H. E. Shortt, Director of the Department of Parasitology and members of the staff for facilities and help. The writer is indebted to Professor B. K. Das of the Osmania University, for providing facilities for the collection of helminths in Hyderabad.

References

Cram, Eloise B., 1927.—" Bird parasites of the nematode sub-orders Strongylata Ascaridata, and Spirurata." *U.S. Nat. Mus. Bull.* 140, *Washington.*

1937.—" A review of the genus *Oxyspirura*, with a morphological study of *O. petrovi* Skrjabin, 1929, recently discovered in galliform birds of the Northern United States." *Papers on Helminthology published in commemoration of the 30 year jubileum of K. J. Skrjabin and of fifteenth anniversary of the All-Union Institute of Helminthology*, pp. 89–98. *Moscow*, 1937.

Seurat, L. G., 1920.—" Sur une Filaire du Heron cendre." *Bull. Soc. Hist. Nat. Afr. N.*, 11, p. 142. (*W.L.* 5131.)

Skrjabin, K. J., 1916.—" Nematodes des oiseaux du Turkestan russe." *Annu. Mus. Zool. Acad. Imp. d'Sci. de Petrograd*, 20, p. 457.

1929.—" Nematoden der Gattung *Oxyspirura* Drasche aus den Augen der Vogel." *Leist. der.* 50. *Helm. Exped.*, 213–234 (1929) *Russisch.*

1931.—" Neue Nematoden der Gattung *Oxyspirura* Drasche aus dem vogelauge." *Z. Parasitenk.* 3. 726–740. (*W.L.* 23536a.)

Sprehn, C. E. W., 1932.—" Lehrbuch der Helminthologie." *Berlin.*

Ssolonitzin, I. A., 1932.—" Ueber Zwei neue Arten von Nematoden im Vogel." *Zbl. Bakt.*, CXXIV (5/6), 361–365. (*W.L.* 23684.)

Stossich, M., 1897.—" Filarie e spiroptere. Lavoro monografico." *Boll. Soc. adriat. Sci. nat.*, XVIII, p. 13. (*W.L.* 3359.)

Yorke, W. & Maplestone, P. A., 1926.—The Nematode Parasites of Vertebrates. *J. A. Churchill, London.*

Journal of Helminthology, Vol. XXII, No. 2, 1948, pp. 93—108.

Some New Nematodes from the Australian Wallaby (*Macropus rufogrisea fruticus*) with a Note on the Synonymy of the Genera *Zoniolaimus*, *Labiostrongylus* and *Buccostrongylus*.

By C. C. Kung, B.Sc.,

Research Assistant in Zoology, Institute of Zoology, Nanking.
(*From the Department of Parasitology, London School of Hygiene and Tropical Medicine.*)

From three wallabies which recently died in the Zoological Gardens, London, a number of nematodes were collected from the stomach and the anterior end of the duodenum. On examination the material proved to contain *Asymmetricostrongylus dissimilis* Nagaty, 1932 (syn. *Trichostrongylus dissimilis* Wood, 1930) and three new species belonging to the genera *Globocephaloides*, *Pharyngostrongylus* and *Zoniolaimus* respectively. The new species are described below under the names *Globocephaloides trifidospicularis* n.sp., *Pharyngostrongylus longibursaris* n.sp., and *Zoniolaimus cobbi* n.sp. Young forms of *A. dissimilis* were found encysted in the gastric mucosa, a hitherto unrecorded occurrence.

A collection of nematodes, labelled "*Labiostrongylus* sp., stomach of *Python spilotus*, Australia, 1933" was examined. The specimens could not be identified as belonging to any of the hitherto described members of the genus *Labiostrongylus*. The name *Labiostrongylus australis* n.sp. is proposed for the new species which is described below. It is doubtful whether the python is the true definitive host—all the hitherto described species of the genus have been from marsupials.

Asymmetricostrongylus dissimilis Nagaty, 1932.
(syn. *Trichostrongylus dissimilis* Wood, 1930.)

The adult parasites were found free in the lumen of the stomach and in one of the wallabies a number of cysts of pin-head size were found embedded in the mucosa of the gastric wall. The cyst wall is white, translucent and thin. Each cyst contained a single male worm which was dissected out and found to represent an exceedingly advanced developmental stage of the parasite. The male genital organs were well differentiated, though less so in comparison with those of the adult. The bursa is well-formed, but the spicules and gubernaculum are not yet completely chitinized. The adults and the encysted forms measure as follows :

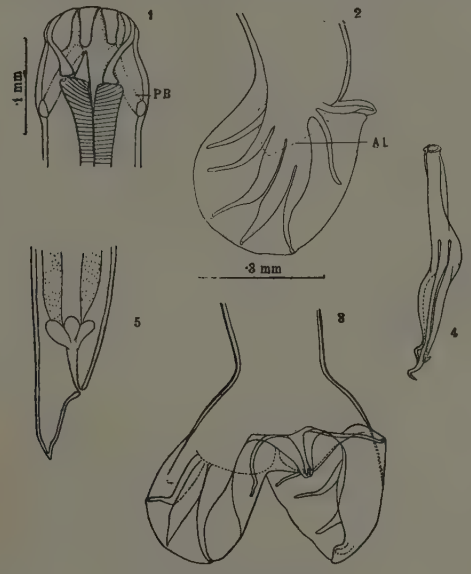
	Adults.	*Encysted forms.*
Body dimensions	6.84-7.2 × 0.1 mm.	6.12-7.56 × 0.095-0.099 mm.
Oesophagus length	0.8-0.92 mm.	0.74 mm.
Spicules	0.22-0.24 mm.	0.215-0.224 mm.
Gubernaculum	0.151 mm.	0.132-0.145 mm.

Though the life-cycle of *Asymmetricostrongylus* sp. has not yet been worked out, one would expect it to be similar to that of *Trichostrongylus*. The encystation of young adult forms in the gastric wall represents a quite unusual phase. As they are believed to have developed from fourth stage larvae, it is conceivable that after the third moult, probably in the alimentary canal, the larvae invade the gastric wall where they cast their sheath (fourth ecdysis) and grow into the young forms as now seen. Finally breaking out from the cyst and the gastric mucosa they regain the lumen of the stomach where they reach sexual maturity.

Globocephaloides trifidospicularis n.sp.

These small worms were found in the anterior end of the duodenum. Some were attached to the intestinal wall. The body generally tends to curve ventrally so that it is dorsally convex; it bends strongly to the ventral side at its anterior end which is gradually attenuated and has conspicuous transverse striations throughout its length. The female measures 13.56-16.06 mm. long by 0.40-0.45 mm. in maximum width; the corresponding dimensions of the male are 8.8-10.5 mm. and 0.32 mm. The mouth is directed anteriorly without being armed; its opening is terminal and simple without the granular circular rings of *Globocephalus*. The buccal capsule is subglobular and averages 0.09 mm. deep by 0.087 mm. wide in the female and 0.077 mm. deep by 0.075 mm. wide in the male. The wall of the buccal capsule is strongly chitinized, the lower portion of the wall being thicker than the roofing portion. The posterior margin is greatly thickened to form a rim articulating with the oesophagus. Six longitudinal cuticular ridges (papillae?) run from the upper half of the wall to the margin of the oral aperture, each ending in a depression; two of them are lateral and comparatively large and the other four are submedian in position. A dorsal (not subventral as described by previous workers) elongated triangular tooth arises from the dorsal sector of the oesophagus and projects 0.031-0.035 mm. into the buccal cavity; four pillar-like bodies (P.B. Fig. 1) arise from the subcuticulum of the body about 0.033 mm. behind the level of the floor of the buccal cavity, or 0.11 mm. from the anterior

end of the body and lie against the buccal wall of the capsule for about half the length of the latter. The oesophagus is slightly club-shaped posteriorly and averages 1.05 mm. and 0.85 mm. long by 0.09 mm. and 0.08 mm. wide in the female and male respectively. The cervical papillae are small and average 0.45 mm. from the anterior end in the



Globocephaloides trifidospicularis n.sp.

Fig. 1. Anterior end of body in lateral view. Fig. 2. Bursa in lateral view. Fig. 3. Bursa in dorso-lateral view. Fig. 4. A spicule. Fig. 5. Female tail in lateral view.

Scale 0.3 mm. refers to Figs. 2, 3, 4 and 5.
P.B., pillar-like body ; A.L., accessory lobe.

female ; the nerve ring is 0.35 mm. and 0.27 mm. and the excretory pore 0.40 mm. and 0.35 mm. from the anterior end in the female and male respectively.

The female : The vulva is salient, usually covered with two large flaps and situated at the junction of the middle and posterior thirds of the body ; the vagina is short and leads into two muscular and divergent ovejectors each of which is followed by a tortuous uterus ; the conical tail is 0.135-0.18 mm. long.

The male: The bursa comprises two large ventro-lateral lobes and a rudimentary dorsal lobe, with highly granular inner surfaces. After giving off at its root the externo-dorsal rays, which are long and slender and run posteriorly, the dorsal ray passes posteriorly, bifurcating into two small short bidigitate branches nearly at the tip; in some specimens the right externo-dorsal ray extends straight outwards instead of posteriorly, thus raising the lateral lobe of the bursa to a remarkable extent; both the ventral and lateral lobes are fused together, resulting in the formation of two large ventro-lateral lobes. Both the externo-lateral and postero-lateral are adjacent and parallel to the medio-lateral proximally for more than one half their length but diverge from it distally; all the laterals are rather thick but greatly reduced in size at about the terminal third, becoming slender and reaching the bursal edge; the ventro-ventral and latero-ventral are long and slender but widely separated from each other. There are two accessory lobes not mentioned before in any known species of this genus. These are short, broad, more or less triangular in shape and are situated under the cover of the dorso-lateral portion of the lateral lobes. The two spicules, equal in length and similar in build, are stout and relatively short (0.50-0.61 mm. in length); the distal half of the spicule is divided into three closely applied branches of which the left one is swollen at its origin and curves outwards, then becomes straight and terminates in a hook; the right one is swollen at its middle and ends in the form of a lancet; the median one is slightly swollen and ends in a terminal hook directed ventrally. This process is slightly longer than the other two. No *gubernaculum* was seen.

This species bears a close resemblance to *G. wallabiae* Johnston and Mawson, 1939 and *G. thetidis* Johnston and Mawson, 1939, and it is difficult to differentiate the new species from the above mentioned ones because they have been described very briefly. But in the present species the spicules are so characteristic that Johnston and Mawson could hardly have failed to comment on them if their species possessed spicules of the same type. They only gave the length of the spicules in *G. thetidis* and stated it to be 0.045 mm. which is apparently a misprint for 0.45 mm. The spicules of *G. wallabiae* are very briefly described without any illustrations. According to their definition of the genus, the posterior extremities of the spicules of Johnston and Mawson's two species seem to be undivided. Furthermore, as previously mentioned, there are two accessory lobes in the bursa of the present species, but no mention was made of the presence of similar structures

in any of Johnston and Mawson's species. However, even if these somewhat doubtful points be disregarded, the present species still differs from *G. wallabiae* in having the vulva at about the junction of the middle and posterior thirds of the body instead of being near the posterior extremity, in the larger size of the body, and in the longer spicules. It differs from *G. thetidis* in the larger dimensions of the buccal capsule, the longer oesophageal tooth, the longer spicules, and in the two large flaps instead of one which cover the vulva.

The relationship of *Globocephaloides* and *Globocephalus*.

Freitas and Lent (1936) regarded *Globocephaloides* Yorke and Maplestone, 1926 as a synonym of *Globocephalus*. Subsequently the genera were reviewed by Johnston and Mawson (1939a) who concluded that they are quite different. The latter view is endorsed by the writer on the basis of the material described above from which the following additional evidence is adduced. In the first place, in all species of *Globocephaloides* the buccal capsule is supported by four pillar-like bodies (P.B. Fig. 1) which are absent in *Globocephalus*. Secondly, in the latter the duct of the dorsal oesophageal gland opens through a conspicuous dorsal gutter in the mid-dorsal line into the buccal cavity, while it does not do so in the former. Thirdly, *Globocephaloides* differs remarkably from *Globocephalus* in the structure of the bursa, particularly in the latero-ventral and ventro-ventral rays which are widely separated in one genus and close together and parallel in the other. Fourthly, as in the new species just described, the spicules of *Globocephaloides* are stout, short and branched, while those of *Globocephalus* are of the ancylostome type, slender and simple. It should be noted also that a gubernaculum is absent in *Globocephaloides*.

Pharyngostrongylus longibursaris n.sp.

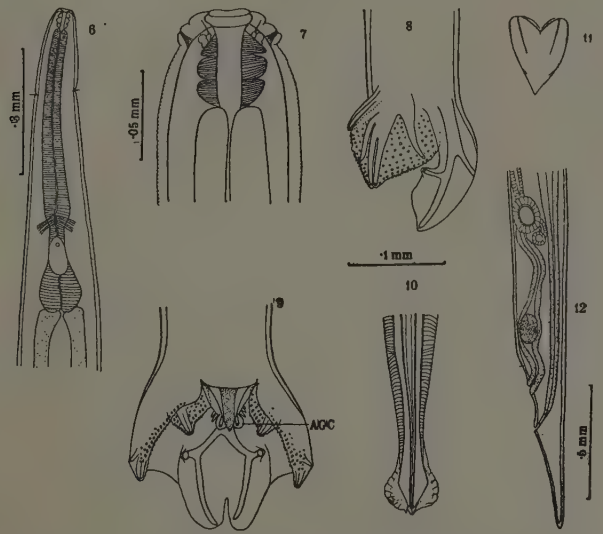
These small worms were found in the stomach. The body has a convex curve dorsally and tapers towards the posterior end. There are fine transverse striations throughout its length except at the posterior end; the female and male measure 7.5-8.9 mm. and 6.8-7.9 mm. long by 0.23-0.31 mm. and 0.23-0.27 mm. wide respectively. The vestibule which is cylindrical in outline with a more or less even diameter throughout its length, is 0.049 mm. long by 0.015 mm. wide (inner wall); the wall is radially striated and subdivided into three sections, as illustrated in Fig. 7. No circumoral lips were seen; the mouth collar has a constriction which gives it a two-ringed appearance; around the base of the

collar, six small papillae are present, two lateral and four submedian in position. Each of the submedian is provided with a seta. The oral aperture is simple and circular. The buccal capsule, ring shaped and shallow, is without a leaf-crown. The oesophagus is of the oxyurid type and measures 0.77 mm. and 0.67 mm. long by 0.067 mm. and 0.063 mm. wide in the female and male respectively; its anterior wide portion, about 0.53 mm. long, is followed by a posterior short narrow portion which ends in a conspicuous bulb. The cervical papillae are filamentous, and situated at 0.227 mm. and 0.213 mm. from the anterior end in the female and male respectively; the nerve ring encircles the junction of the two portions of the oesophagus and the conspicuous excretory pore is situated a little behind it, 0.667 mm. and 0.59 mm. from the anterior end in the female and male respectively.

The female: The vulva and anus are 0.66 mm. and 0.46 mm. respectively from the tip of the tail which is long and tapers to a fine point; the vagina is more or less meandering, 0.7-0.8 mm. long; the uteri are parallel.

The male: The bursa is large in comparison with the body and all the lobes are distinctly separated from each other; the lateral lobes are large and broad; the dorsal lobe is especially prominent and is deeply indentated in the median line; the ventrals are comparatively small and separated from each other; the inner surfaces of the lateral and ventral lobes are papillated and have fringed edges. The dorsal ray is stout, passes posteriorly for a distance and bifurcates at the level of the end of the genital cone into two long branches, each passing posteriorly and laterally to the bursal edge; a lateral branch arises from each of the branches at the anterior third of its length after bifurcating, extends laterally and ends in a raised ridge on the surface of the dorsal lobe; the externo-dorsals arise from the common trunk with the laterals, pass posteriorly and diverge dorsalwards from the laterals; the ventrals are close together, equal and similar. The genital cone is prominent and extends posteriorly as far as the posterior edges of the ventral lobes, usually with an appendage on each side (A.G.C. Fig. 9). The two slender spicules are equal in length, 1.07 mm. long, similar in build, bearing striated alae, at their tips the alae expand to form a fan-like membrane which holds the spicules together; the tips appear united distally. The gubernaculum is weakly chitinized and heart-shaped when viewed dorsally or ventrally and measures 0.08 mm. long by 0.057 mm. wide. The prebursal papillae are small and situated 0.25 mm. from the tip of the dorsal lobe.

This species is very like *P. zeta* Johnston and Mawson, 1939, but differs from it in the shape of the vestibule and particularly in its three sharply distinct divisions; in the longer dorsal lobe of the bursa; in the relatively posterior position of the cervical papillae, nerve ring and excretory pore; in shorter spicules and the presence of the fan-like membrane enclosing the terminal portions of the spicules. This species possesses a gubernaculum of which no mention was made in the description of *P. zeta*. These characters warrant the erection of a new species.



Pharyngostrongylus longibursaris n.sp.

Fig. 6. Anterior portion of body in ventral view. Fig. 7. Anterior end of body in dorsal view, highly magnified. Fig. 8. Bursa in lateral view. Fig. 9. Bursa in ventral view. Fig. 10. Terminal ends of two spicules in dorsal view, showing fan-like membrane enclosing them. Fig. 11. Gubernaculum in dorsal view. Fig. 12. Female tail in lateral view.

Scale 0.1 mm. refers to Figs. 8, 9, 10 and 11.
A. G. C, appendage of genital cone.

So far as I am aware, 16 species of this genus including the present new one have been reported from marsupials. In the course of this study it was observed that with the exception of some doubtful ones they fall into two sharply-defined groups, namely those possessing

leaf-crown, long vestibule (pharynx) and cervical papillae in the vestibular region; and those without a leaf-crown but having a short vestibule and the post-vestibular cervical papillae. The first group is represented by *P. macropodis* and *P. gamma*. *P. eta* and *P. iota*, though conforming to the group characters, are, however, left in a doubtful position because *P. eta* possesses a pair of intestinal caeca around the base of the oesophagus which moreover is without a posterior bulb and *P. iota* is without any striations on the vestibular wall. Though *P. brevis* possesses a rudimentary leaf-crown, it appears to belong with the remainder to the second group on account of the other features as a whole. In view of the inadequacy of the descriptions of some species as well as the inaccessibility of the type specimens for examination, it is not advisable to separate this genus into new genera at the present time.

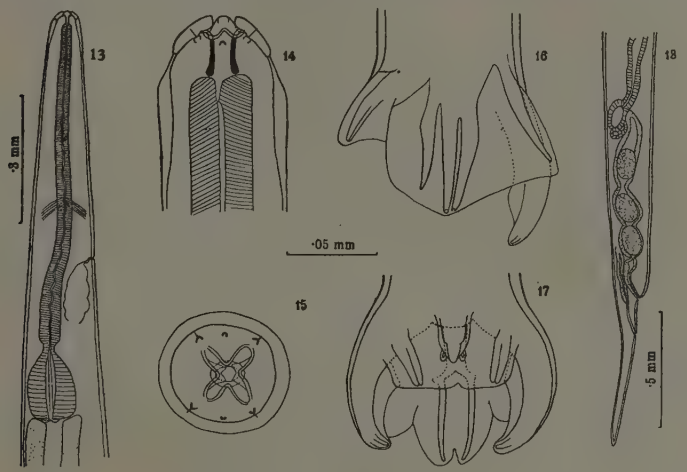
Zoniolaimus cobbi n.sp.

These small slender worms were found in the stomach. The body tapers towards the posterior end and is provided with fine transverse striations throughout its length. It is 9.9-10.2 mm. and 7.8-8.7 mm. long by 0.25 and 0.19-0.22 mm. wide in the female and male respectively; at the anterior end there are four small lip-like processes, one dorsal and one ventral, each with an indentation at the tip, and two laterals conical in shape; there are six papillae at the base of the processes, four being submedian, each with a forwardly directed seta, and two lateral in position; the oral aperture is simple and circular; the buccal capsule is cylindrical, 0.027 mm. long by 0.01 mm. wide, apparently without a leaf-crown. The oesophagus is 0.79-0.86 mm. and 0.83-0.87 mm. long by 0.047-0.053 mm. and 0.05-0.058 mm. wide in the male and female respectively. It is of the oxyurid type, consisting of an anterior long portion suddenly constricted and followed by a conspicuous bulb about 0.153 mm. long by 0.099 mm. in diameter; the nerve ring is 0.37-0.45 mm. from the anterior end; the excretory pore is conspicuous and a little behind the nerve ring; and the cervical papillae are filamentous, 0.532-0.62 mm. from the anterior end.

The female: The vulva is 0.762 mm. from the tip of the tail which is 0.57 mm. long, slender and tapers to a fine point; the vagina is 0.85 long and the uteri are parallel.

The male: The bursa consists of distinctly separated lobes of which the laterals are large and broad, the dorsal slightly longer than

the others and the ventrals small and separated from the others but fused together to form a broad lobe covering the ventral side. Generally speaking, the bursal pattern is very similar to that of *Pharyngostrongylus*. The dorsal ray is stout and bifurcates at about the level of the ventral bursal edge into two branches each of which immediately gives off a very short stump. The two branches pass posteriorly to the bursal edge and remain parallel to each other; the externo-dorsals arise



Zoniolaimus cobbi n.sp.

Fig. 13. Anterior portion of body in lateral view. Fig. 14. Anterior end of body in lateral view. Fig. 15. End-on view of anterior end of body. Fig. 16. Bursa in lateral view. Fig. 17. Bursa in ventral view. Fig. 18. Female tail in lateral view. Scale 0.05 refers to Figs, 14, 15, 16, and 17.

separately from the laterals and extend to the bursal edge; the mediolateral and postero-lateral are close together, parallel to each other, equal in length, and reach the bursal edge; the externo-laterals are close and parallel proximally to the laterals but diverge distally from them; the ventrals lie close together, are similar and equal and nearly reach the bursal edge. There are two similar equal spicules, 2.29-2.67 mm. long; they are slender, with striated alae, and end in a point with their tips firmly held together by a membrane. The gubernaculum is very weakly chitinized and similar to that of *Pharyngostrongylus longibursaris* except that it is much smaller, being only 0.046 mm. long by 0.037 mm. wide. The genital cone is conspicuous. The prebursal papillae

are small and situated 0.133-0.178 mm. from the tip of the dorsal lobe of the bursa.

This species is allied to *Z. australis* (syn. *Buccostrongylus australis* Johnston and Mawson, 1939) but it possesses only four small lip-like processess instead of six; its spicules are twice as long as those of *Z. australis,* and the ventral lobes are fused together whereas they are separated from each other in the latter. These characters appear to justify the creation of the new species, *Z. cobbi.*

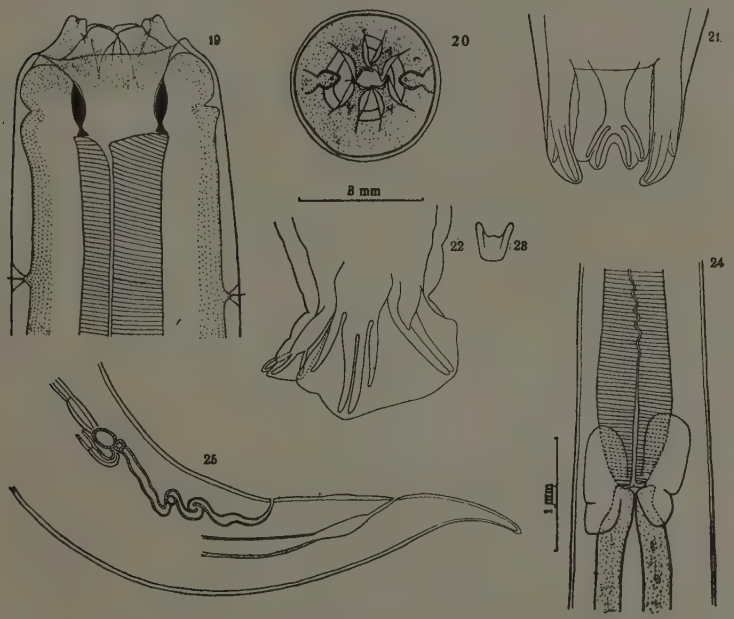
Labiostrongylus australis n.sp.

These are stout worms recorded probably in error from the stomach of *Python spilotus*. The body which has extremely fine transverse striations is 36-38 mm. and 22.7-27.5 mm. long by 1.57-1.61 mm. and 1.03-1.11 mm. wide in the female and male respectively; the anterior end bears six prominent lip-like processes, four submedian, and two lateral. The submedians are large and rectangular, each with a papilla (bearing a short seta) half way along its length. The laterals are spindle-shaped and a little longer than the submedians when viewed laterally, with a papilla at the distal end. The buccal capsule is about 0.125 mm. long by about 0.17 mm. wide at the base of the cavity. The oesophagus is slightly club-shaped, 6.45-6.75 mm. and 4.85-5.0 mm. long by 0.667 mm. and 0.533 mm. wide in the female and male respectively. Two intestinal diverticula extend forwards as well as a little backwards, forming two pouch-like bodies around the base of the oesophagus; the cervical papillae are filamentous, 0.55 mm. from the anterior end; the nerve ring is 1.4-1.6 mm. and 0.99-1.09 mm. and the excretory pore 1.79-1.95 mm. and 1.25-1.48 mm. from the anterior end in the female and male respectively.

The female: The vulva is 2.26-2.3 mm. and the anus 1.14-1.17 mm. from the tip of the tail; the tail is stumpy and turns back dorsally to terminate in a point. The vagina is 2.09-2.39 mm. long and the uteri are parallel.

The male: The bursa is small and short in comparison with the body and consists chiefly of lateral and ventral lobes fused together, from which the dorsal lobe is separated. The ventral rays are close together, parallel, long and equal, pass straight down nearly to the bursal edge and are directed ventralwards; the postero- and medio-laterals are close together and parallel to each other, long and equal, reaching the bursal edge; the externo-laterals are close and parallel to the medio-laterals proximally but a little divergent from them distally.

The dorsal ray is stout, passes posteriorly for a considerable distance before giving off two long lateral branches and immediately two long terminal branches, all the four branches being subequal. The two spicules are equal and similar, 7.95-8.57 mm. long with striated alae and terminate in a fine point. The gubernaculum is 0.082 mm. long by 0.067 mm. in maximum width. The genital cone is prominent.



Labiostrongylus australis n.sp.

Fig. 19. Anterior end of body in dorsal view. Fig. 20. End-on view of anterior end of body. Fig. 21. Bursa in dorsal view. Fig. 22. Bursa in lateral view. Fig. 23. Gubernaculum in dorsal view. Fig. 24. Oesophagus-intestinal junction. Fig. 25. Female tail in lateral view.

Scale 0.3 mm. refers to Figs 19, 20, 21, 22 and 23.
Scale 1.0 mm. refers to Figs. 24 and 25.

This species resembles *L. communis* (syn. *Zoniolaimus communis* Johnston and Mawson, 1939) and *L. clelandi* (syn. *Z. clelandi* Johnston and Mawson, 1939). But it differs from the former in having much longer spicules, in the fusion of the ventral lobes of the bursa with the laterals and in the position of the papillae on the submedian lips.

The new species has a gubernaculum but no mention is made of this structure in *L. communis*. It differs from *L. clelandi* in that the four submedian cephalic papillae are each provided with a short seta. In *L. clelandi* the submedian papillae appear to be lacking but long setae are present. It has a shorter dorsal lobe and a different disposition of the bursal rays, particularly that of the dorsal ray which gives off two pairs of long terminal branches arising close together from the common trunk. In *L. clelandi* the dorsal ray gives off a fairly long branch on each side, the main stem continuing for a distance somewhat greater than the length of each branch and then bifurcating into two terminal branches. Moreover, the ventro-ventral and externo-ventral rays are fused together for their proximal half in *L. clelandi*, whereas they are adjacent and parallel throughout their whole length in the new species. Finally, the new species possesses two intestinal diverticula and a gubernaculum, which are lacking in the description of *L. clelandi*.

The relationship of *Zoniolaimus* Cobb, 1898, *Labiostrongylus* Yorke and Maplestone, 1926, and *Buccostrongylus* Johnston and Mawson, 1939.

The genus *Zoniolaimus* was created by Cobb (1898) for his two species, *Z. setifera* and *Z. brevicaudatus* from *Macropus* sp. but no descriptions were given other than some drawings and his formula. Johnston and Mawson (1939b), however, published enlargements of Cobb's drawings with a description of the male *Z. setifera* and briefly described, without any illustrations, both sexes of *Z. brevicaudatus*. Since 1898, no species had been added to the genus until 1939 when Johnston and Mawson described six new species from Australian marsupials in Queensland, namely: *Z. bancrofti*, *Z. bipapillosus*, *Z. communis*, *Z. insularis*, *Z. onychogale*, and *Z. uncinatus*. In the same year (1939b) two more species, *Z. clelandi* and *Z. ualabatus*, were described by them from marsupials in New South Wales. In 1940 they recorded another new species, *Z. eugenii*, from Australian marsupials.

The genus *Labiostrongylus* was erected by Yorke and Maplestone (1926) for a single species *L. labiostrongylus*, from *Macropus* sp. In 1929, Wood added a new species, *L. longispicularis*, to this genus, and in 1938 Johnston and Mawson recorded three further new species from marsupials, namely, *L. macropodis*, *L. grandis*, and *L. petrogale*. On examining a number of species which are believed by them to be

congeneric with *Zoniolaimus* the latter authors (1939a) treated *Labiostrongylus* Yorke and Maplestone as a synonym of it and transferred all species formerly belonging to *Labiostrongylus* into *Zoniolaimus*.

The genus *Buccostrongylus* was created by Johnston and Mawson (1939a) for the reception of the two species, *B. australis* and *B. buccalis*, from marsupials. The chief characteristics of this genus are a long cylindrical buccal capsule, inconspicuous lip-like processes, a long slender oesophagus which ends in a distinct bulb, the separation of the externo-dorsal rays from the laterals of the bursa, and a long slender tail in the female. In the same year (1939b) two more species, *B. labiatus* and *B. setifer*, were added to the genus by the same authors.

In the writer's opinion, a re-arrangement of many species of the above three genera is necessary. Thus, *Zoniolaimus setifera* and probably also *Z. brevicaudatus* should be congeneric with at least three of the four species at present occupying the genus *Buccostrongylus*. The reasons for this view are as follows. Firstly, they are small slender worms, usually less than 10 mm. long by less than 0.26 mm. wide. Secondly, the buccal capsule is cylindrical and its length is greater than the width. Thirdly, they have small and inconspicuous circumoral lip-like processes. Finally, the oesophagus is of the oxyurid type, consisting of an anterior long, wide portion and a conspicuous large bulb posteriorly with a pronounced constriction between them. With regard to the morphology of the bursa, while it is true that the disposition of the externo-dorsal rays in *Buccostrongylus* differs from that of *Z. setifera*, as illustrated by Cobb, this can hardly be regarded as a character of generic rank, since it not infrequently happens that helminths belonging to obviously different genera possess bursae of a very similar pattern. The difference indicated above, does not therefore materially weaken the case for the fusion of *Zoniolaimus* and three species of *Buccostrongylus*. As regards the characteristics of the caudal extremity of the females it is impossible to establish or disprove any relationship because no particulars about the characteristics of the females appear in either Cobb's paper or Johnston and Mawson's descriptions.

The remaining species of *Zoniolaimus* should be transferred to the genus *Labiostrongylus* on the basis of the following morphological affinities. Firstly, they are stout large worms usually 25 mm. long by 1.1 mm. thick. Secondly, their buccal capsules are shallow and wider than long. Thirdly, they have very prominent and rather large circumoral lip-like processes. Finally, the oesophagus is club-shaped,

sometimes with intestinal diverticula at the oesophago-intestinal junction.

The result of this re-allocation of species is that the genus *Buccostrongylus* would retain only one species, the rather doubtful *B. setifer* Johnston and Mawson, 1939. *Zoniolaimus* would include *Z. setifera* Cobb, 1898; *Z. brevicaudatus* Cobb, 1898; *Z. australis* (syn. *B. australis* Johnston and Mawson, 1939); *Z. buccalis* (syn. *B. buccalis* Johnston and Mawson, 1939); *Z. labiatus* (syn. *B. labiatus* Johnston and Mawson, 1939) and *Z. cobbi* n.sp. *Labiostrongylus* would comprise the following: *L. labiostrongylus* Yorke and Maplestone, 1926; *L. longispicularis* Wood, 1929; *L. macropodis* Johnston and Mawson, 1938; *L. grandis* Johnston and Mawson, 1938; *L. petrogale* Johnston and Mawson, 1938; *L. bancrofti* (syn. *Z. bancrofti* Johnston and Mawson, 1939); *L. bipapillosus* (syn. *Z. bipapillosus* Johnston and Mawson, 1939); *L. communis* (syn. *Z. communis* Johnston and Mawson, 1939); *L. insularis* (syn. *Z. insularis* Johnston and Mawson, 1939); *L. onychogale* (syn. *Z. onycholgale* Johnston and Mawson, 1939); *L. uncinatus* (syn. *Z. uncinatus* Johnston and Mawson, 1939); *L. clelandi* (syn. *Z. clelandi* Johnston and Mawson, 1939); *L. ualabatus* (syn. *Z. ualabatus* Johnston and Mawson, 1939); *L. eugenii* (syn. *Z. eugenii* Johnston and Mawson, 1940) and *L. australis* n.sp.

Definition of *Zoniolaimus*.

The definition of *Zoniolaimus* given by Johnston and Mawson (1939) is actually that of *Labiostrongylus* because it was based mainly on that given for the latter genus by Yorke and Maplestone (1926). Yorke and Maplestone's generic diagnosis of *Labiostrongylus*, therefore, stands but the genus *Zoniolaimus* Cobb (1898) remains to be defined. The following diagnosis is partly based on that of *Buccostrongylus* given by Johnston and Mawson (1939).

Trichoneminae—Small worms. Mouth directed forwards and surrounded by four to six (eight?) small lip-like processes at the base of which as a rule, there are six small papillae, four being submedian and two lateral in position. There is no leaf-crown. Buccal capsule is cylindrical, its length greater than the width and chitinized. Oesophagus is long and slender, of oxyurid type with a well defined posterior bulb.

Male: Bursa small with separated lobes; dorsal ray after one-third or one-half of its length bifurcating into two long branches. The externo-dorsal arises from the base of the laterals but is divergent from

them; the medio- and postero-laterals are close and parallel; the externo-laterals arise from the common trunk with the laterals; the ventrals are close and parallel; two spicules equal and similar; gubernaculum usually present; genital cone present.

Female: The tail is long and pointed; vulva a little distance in front of anus; vagina long and uteri parallel; oviparous. Parasites in alimentary canal of marsupials. Type species: *Z. setifera* Cobb, 1898.

Summary.

1. The present paper consists of descriptions of an encysted developing stage of *Asymmetricostrongylus dissimilis*, and of four new species, *Globocephaloides trifidospicularis*, *Pharyngostrongylus longibursaris*, *Zoniolaimus cobbi* and *Labiostrongylus australis*. With the exception of *L. australis*, which is doubtfully recorded from *Python spilotus*, all the species came from the alimentary canal of the Australian wallaby, *Macropus rufogrisea fruticus*.

2. The validity of the genus *Globocephaloides* is strengthened by some new data.

3. The relationship of *Zoniolaimus*, *Labiostrongylus* and *Buccostrongylus* is considered and in consequence a re-arrangement of the species within these genera is proposed.

Acknowledgments.

I should like to take this opportunity to express my sincere gratitude to Professor J. J. C. Buckley for his constant advice and criticism during the course of the work. Many thanks are also due to Professor H. E. Shortt, Director of the Department of Parasitology, for facilities to carry out the work in his Department, and to Dr. P. L. Leroux for his great help in the collection of the material.

References

Canavan, W. P., 1931.—" Nematode parasites of vertebrates in the Philadelphia Zoological Gardens and vicinity II." *Parasitology*, XXIII (2), 196–228. (*W.L.* 16035.)

Cobb, N. A., 1898.—" Extract from MS report on the parasites of stock." *Agric. Gaz., N.S.W.*, IX, pp. 296 & 419. (*W.L.* 278.)

de Freitas, J. F. T. & Lent, H., 1936.—" Estudo sobre o genero Globocephalus Molin, 1861." *Mem. Inst. Osw. Cruz.*, XXXI (1), 69–79. (*W.L.* 13465.)

JOHNSTON, T. H. & MAWSON, P. H., 1938.—" Strongyle nematodes from Central Australian kangaroos and wallabies." *Trans. Roy. Soc. S. Aust.*, LXII (2), 263–286. (*W.L.* 21654.)

1939a.—" Strongyle nematodes from Queensland marsupials." *Trans. Roy. Soc. S. Aust.*, LXIII (1), 121–148. (*W.L.* 21654.)

1939b.—" Strongyle nematodes from marsupials in New South Wales." *Proc. Linn. Soc. N.S.W.*, LXIV (5/6), 513–536. (*W.L.* 16790a.)

1940.—" On a collection of nematodes from Australian marsupials." *Rec. Aust. Mus.*, XX (5), 360–366. (*W.L.* 17736.)

1940.—" A key to the nematode parasites of Australian marsupials and monotremes." *Trans. Roy. Soc. S. Aust.*, LXIV (2), 262–370. (*W.L.* 21654.)

1942.—" The Gallard collection of parasitic nematodes in the Australian Museum." *Rec. Aust. Mus.*, XXI (2), 110–115. (*W.L.* 17736.)

MÖNNIG, H. O., 1926.—" The life histories of *Trichostrongylus instabilis* and *T. rugatus* of sheep in S. Africa." *11th & 12th Rep. Dir. Vet. Res. S. Afri.*, 1926–1927. (*W.L.* 18640.)

1926.—" Three new helminths." *Trans. Roy. Soc. S. Afri.*, XIII (3), 291–298. (*W.L.* 21653.)

NAGATY, A. F., 1938.—" The genera *Asymmetricostrongylus* Nagaty, 1932, and *Libiostrongylus* Lane, 1923 and their relation to genus *Trichostrongylus* Loose, 1905." *Livro Jubilar do Prof. Travassos*, Rio de Janeiro, Brazil III.

WOOD, W. A., 1929.—" On a new species of Labiostrongylus." *Ann. Mag. Nat. Hist.*, IV, Seri 10. 550–551. (*W.L.* 1050.)

1930.—" Some new parasitic nematodes from W. Australia." *1st. Rep. Div. Ani. Patho. Inst. Camb. Uni.*

YORKE & MAPLESTONE, 1926.—The nematode parasites of vertebrates. J. & A. Churchill, London.

Journal of Helminthology, Vol. XXII, No. 2, 1948. pp. 109—116.

The Galls caused by *Anguillulina balsamophila* (Thorne) Goodey on the leaves of *Wyethia amplexicaulis* Nutt. and *Balsamorhiza sagittata* Nutt.

By J. Basil Goodey, B.Sc.
(*Nematology Department, Rothamsted Experimental Station.*)

The gall-forming nematode, *Anguillulina balsamophila* (Thorne) Goodey has been described by Thorne (1926) who figures a photograph of a galled leaf of *Balsamorhiza sagittata*. Thorne gives a brief description of the superficial appearance of the galled leaves and mentions that the heavily infested plants are often very stunted. He recorded the parasite on three hosts, two species of Balsam-root, *Balsamorhiza sagittata* Nutt., *B. macrophylla* Nutt. and Mule-ear, *Wyethia amplexicaulis* Nutt. Later Steiner and Buhrer (1933) reported the parasite from the annual sunflower, *Helianthus annuus* L. collected and identified by W. D. Courtney. The detailed structure, however, of the gall has not so far been described and as suitable material was to hand, this paper is the result of some observations made on the gall structure.

Briefly the life history is that early in the growing season the infective larvae enter the young leaves whilst they are still forming in the crowns. By the time the petioles have developed the larvae are no longer able to make an entry into the leaves. Usually a group of larvae enter at each position where a gall will form. This original point of entry is later visible as a small depression on the surface of the mature gall. After about three weeks from entry eggs are laid which hatch to larvae 0.35-0.4 mm. long, these develop rapidly so that by mid-May they are about 1 mm. long. These larvae undergo no further change and become quiescent when the leaves wither and die in July and August. During the winter the plants become crushed by the snow and the leaves decay; in the spring the larvae escape and congregate round the developing crowns.

Material and Methods.

The material was collected by Dr. T. Goodey in June 1947 near to Salt Lake City, Utah, U.S.A. It consisted of leaves of *Wyethia amplexicaulis* pickled in 4% formalin from Parley Canyon, Salt Lake City, and herbarium specimens of the leaves of *Balsamorhiza sagittata* from the type locality, Wellsville Divide, Utah.

The herbarium material was soaked in absolute alcohol for 24 hours and then gradually brought down to water, keeping the specimens in the embedding oven; the process took about six days. Pieces of both normal and galled leaves were taken through dioxan and embedded in paraffin with the addition of one-tenth part of white beeswax, Grigg (1938). This wax has considerable solidity and obviates difficulties associated with the use of high temperatures as it is possible to infiltrate at 47°F. Sections were cut at 10μ and stained by the Safranin and Light Green method. Chlorazol Black E, Green and Blue were also used, Cannon (1941).

Wyethia amplexicaulis.

Normal leaf. The normal leaf (Fig. 1a) is about 0.4 mm. thick bounded on either surface by an epidermis with a slight cuticle and bearing occasional small knob-like hairs. The mesophyll is of a simple nature with no differentiation into palisade and spongy parenchyma and it contains a fairly normal amount of intercellular air-space. The smaller veins are simple collateral ones whilst the larger bundles are surrounded by a layer of thin-walled fibres with lignified walls. Extending above and below the vascular tissue are patches of collenchyma but slightly thickened. Stomata occur in both epidermides but are not numerous.

The Gall. Most of the galls (Fig. 1b) are about 2-4 mm. in diameter and between 1 and 2 mm. in depth. The structure is relatively simple though it shows considerable proliferation of the leaf tissues. The centre of the gall is hollow and is surrounded by a region of cells with dense protoplasmic contents. These cells are, for the most part, non-vacuolate and a prominent feature is the large nucleus with sometimes one or two distinctive nucleoli. These cells are slightly smaller than the normal mesophyll cells and are packed closely together with no intercellular space system. The galls involve vascular tissue in their structure and this vascular tissue extends laterally around the inner zone of cells. There are patches of fibrous cells extending from the vascular tissue to the epidermis in various places consisting mostly of collenchyma.

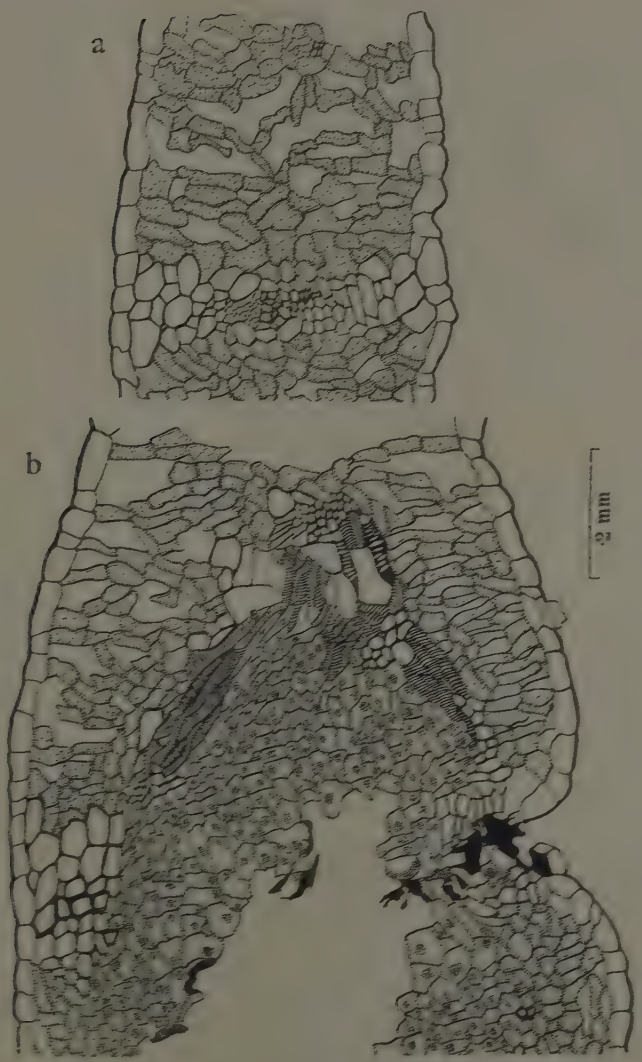


Fig. 1. *Wyethia amplexicaulis*. (a) T. S part of normal leaf. (b) T.S. half a gall showing point of entry of the worms.

There are also patches of sclerenchymatous cells usually more closely associated with the bundles and also occasional isolated stone-cells. The epidermis is unaltered and again stomata are infrequent. The cavity of the gall is irregular and contains numerous worms mostly larvae though an occasional adult is seen. The bounding surface of the cavity shows a collapsed cell here and there along the irregular surface. At one point, either on the upper or lower surface of the gall a slight depression can usually be seen, it is often brownish in colour. A section through the gall at this point shows it to be the point of entry of the nematodes at their original infection of the leaf. The path of entry becomes blocked with a mass of material which stains deeply and the cells immediately around the area are either partially collapsed or have very dense contents. Some of the cells surrounding this area have slightly thickened walls and are collenchymatous in nature.

Balsamorhiza sagittata.

Normal Leaf. The normal leaf (Fig. 2a) appears to be about 0.3 mm. thick and is covered with a dense layer of fine hairs. There is a slight differentiation of the mesophyll so that the uppermost tissue is an indefinite palisade layer. The rest of the mesophyll has an extensive intercellular space system. The bundles are normal collateral ones, the larger ones having patches of collenchyma above and below stretching from the bundle to the epidermis, the largest bundles have in addition, an upper and lower patch of pericyclic fibres. The stomata are numerous in both epidermides and are raised slightly above the general epidermal level.

The Gall. The galls (Fig. 2b) project mostly from the lower surface of the leaf and are about 3-4 mm. in diameter. Sometimes when two are adjacent their cavities become confluent. The cavity of the gall is not very regular and is surrounded by a zone of cells about three or four layers thick. Some of the cells of the surface layer of this zone have collapsed and often their contents stain very deeply. This inner zone is more or less completely surrounded by a layer of vascular tissue with both tracheids and phloem elements stretching round the gall in all directions. Outside this is assimilatory tissue also showing slight differentiation into palisade and spongy mesophyll. The epidermis has a much more thickened cuticle than the normal leaf and in places may be more than one cell thick; it has normal stomata and hairs. In the accompanying figure where a lower quadrant of a section of a gall is

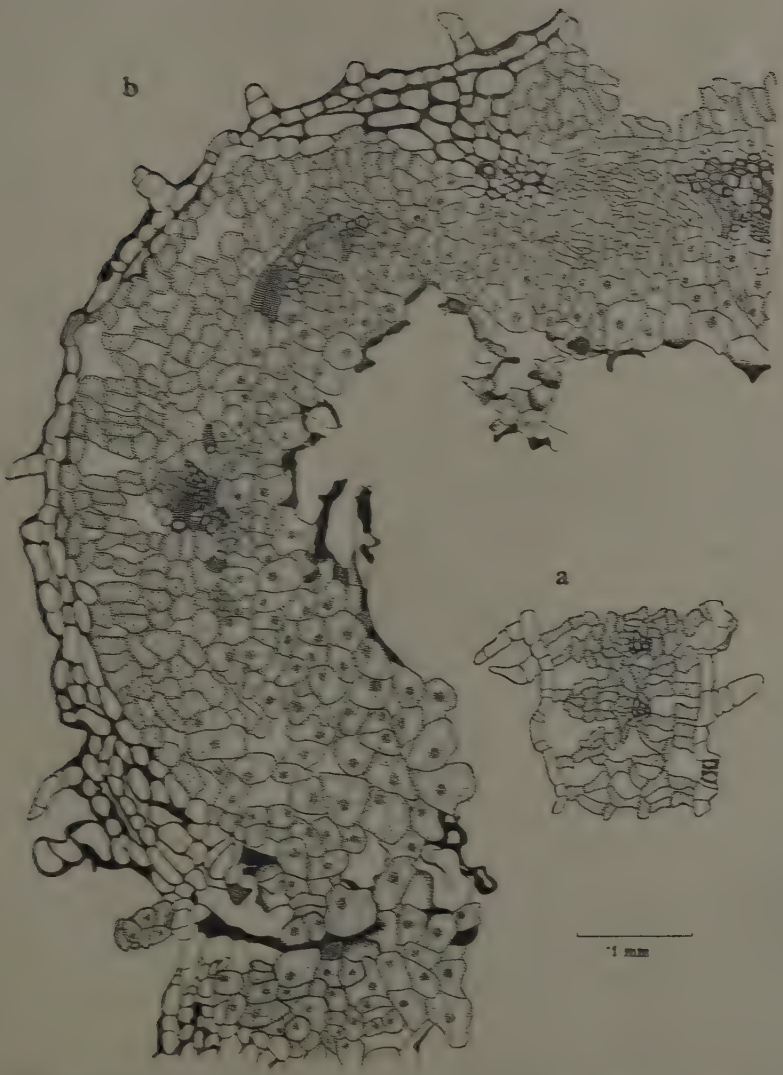


Fig. 2. *Salsuriella salsuria*. (a) T.S. part of normal leaf. (b) T.S. lower quadrant of a gall showing point of entry of the worms.

shown, the increased collenchymatous tissue and generally disturbed conditions round the original point of entry are clearly seen. The path of entry is blocked by densely staining material, some adjacent cells have denser contents while others are very thin-walled and have no contents nor do they stain at all. The general impression given by the gall tissue is that the cells composing it are all slightly larger than those of the normal parts of the leaf. The gall cavity contains numerous worms, mostly larvae though a few adults are also present.

A comparison of the galls on *Wyethia* and *Balsamorhiza* shows some similarities and some differences. Each has an inner zone of cells with dense protoplasmic contents and some of the innermost bounding cells collapsed. This zone is more or less encircled by a layer of vascular tissue, in its turn surrounded by assimilatory tissue. In both there is increased fibrous tissue mostly of a collenchymatous nature. The main difference lies in the relative size of the cells composing the inner zone of tissue; in *Wyethia* these cells are slightly smaller than cells in normal parts of the leaf but in *Balsamophila* the opposite is the case.

Discussion.

It now appears to be generally accepted that the stimulus for the formation of galls is of a chemical nature and, in that growth is the outcome of the stimulus, auxins play some part in the story. Went and Thiman (1937) p. 248 say: "There are a number of diseases in which growth correlations are destroyed or new ones established and in these auxin must play a part. Galls may be due to the production of growth hormones by the infective agent. Indeed, both Sachs (1882) and Beijerinck (1897) based their views on growth correlations partly on such pathological growths. In a study of *Erineum* outgrowths on leaves (intumescences), La Rue (1935, 1936) has shown that auxin will produce similar effects, while Brown and Gardner (1936) have shown the same thing for crown gall. In the case of root nodules there is good evidence that they are due to auxin produced by the infective agent, *Rhizobium leguminosarum*". Also Kostoff and Kendall (1929) with regard to Cynipid galls say that the galls are the "reaction product of the plant tissue dependent on:

1. Plant specificity, including the amount of normal protective substances in the plant and the plant potency of acquiring protective substances against invading foreign substances.

2. The quantity of sap entering the organ or tissue where the gall originates.

3. The specificity of the insect, which one considers in the quality of the foreign substances (excretions) originating from this source.

4. The quantity of the foreign substances.

5. Mechanical injuries and their disintegration products.

6. The presence of unicellular organisms and the activity of their products, qualitative and quantitative.

The writer (1939) discussed the question of the formation of galls due to *Anguillulina dipsaci* in *Plantago lanceolata* and similar arguments can be adduced for the formation of these *A. balsamophila* galls. The galls are more elaborate than those in *P. lanceolata* but the hosts of *A. balsamophila* manifest greater diversity of tissues than is to be seen in the Plantain and, of course, a different parasite is concerned. The inner zone of cells with dense contents has been called the "nutritive" zone by Kostoff and Kendall (l.c.) and indeed, it serves this purpose for the collapsed cells to be found lining the gall cavity are in all probability the result of puncture by the nematode in its feeding. The use of the stylet by *A. dipsaci* in feeding has been reported by Linford (1937), and it is probably a fair speculation that *A. balsamophila* feeds in a similar fashion. The plexus of vascular tissue surrounding this "nutritive" zone ensures that adequate supplies of nutriment are conveyed to the zone. The case can be looked upon from another angle suggested by the observations of Quanjer (1927) on the isolation of necrotic areas in the potato by means of renewed meristematic activity. The gall is then the outcome of the plant's reaction to isolate the infecting organism. The "nutritive" zone shows a character of meristematic tissue namely the dense cell contents with very little vacuolation, so that here also can be advanced a reason for the liberal supply of vascular tissues to the zone.

On the basis of somewhat slender circumstantial evidence the hypothesis that nematode galls are caused by excretions of a similar nature to auxins may be suggested but it still remains to be demonstrated that growth hormones are produced by nematodes.

References.

Goodey, J. B., 1939.—" The Structure of the Leaf Galls of *Plantago lanceolata* L. induced by *Anguillulina dipsaci* (Kühn) Gerv. and v. Ben." *J. Helm.* XVII (4). 183–190. (*W.L.* 112246.)

GRIGG, F. C., 1938.—" An Improved Paraffin Wax for Embedding." *J. Quekett micr. Cl.* I, 4th ser., 4–11. (*W.L.* 11479.)

KOSTOFF, D. & KENDALL, J., 1929.—" Studies on the Structure and Development of Certain Cynipid Galls." *Biol. Bull. Wood's Hole.* LVI (6), 402–458. (*W.L.* 2975.)

LINFORD, M. B., 1937.—" Notes on the feeding of *Ditylenchus dipsaci* (Nematoda ; Tylenchidae)." *Proc. Helminth. Soc. Wash.* IV (2), 46–47.

QUANJER, H. M., 1927.—" Een aaltesziekte van de aardeappelplant, de aantastingawijze en de herkomst van haar oorzak, *Tylenchus dipsaci* Kühn." *Tijdschr. PlZiekt.*, XXXIII, 137–172. (*W.L.*21280.)

STEINER, G. & BUHRER, E. M., 1933.—" Recent Observations on Diseases caused by Nematodes." *Plant Disease Reporter.* XVII (14), 172–3.

THORNE, G., 1926.—" *Tylenchus balsamophilus*, a New Plant Parasitic Nematode." *J. Parasit.* XII, 141–145. (*W.L.* 11428.)

WENT, F. W. & THIMAN, K. V., 1937.—*Phytohormones*, New York.

Journal of Helminthology, Vol. XXII, Nos. 3/4, 1948. pp. 117—127

Potato Root Eelworm, D-D, and Soil Sterilization. I. Methods and Criteria.

By B. G. Peters, M.Sc., Ph.D.

(*Nematology Department, Rothamsted Experimental Station, Harpenden*).

In the interpretation of field experiments on the use of D-D for controlling the potato root eelworm, *Heterodera rostochiensis*, various difficulties have arisen. The chemical seemed far less effective in killing the eelworm than might have been expected from results published in the U.S.A., yet on the other hand greater increases in the yield of potatoes were produced than could reasonably be explained by the small proportion of eelworms killed. The whole situation is suggestive of a partial sterilization effect, such as was found in the early work at Rothamsted and elsewhere when volatile organic compounds were injected into soil. In Hawaii, Carter and his colleagues have noted a similar "soil amendment" effect from the injection of D-D.

Whilst this effect is an important factor on the credit side, when assessing the results of D-D injections, it is to be sharply differentiated from the purely nematocidal effect. There must be many organic substances which would give comparable soil amendment effects, reflected in increased yields, without killing eelworms at all, and their continued use could only lead to an increase in the eelworm population, possibly to disastrous levels. It follows that yields cannot be used as a criterion of nematocidal efficacy in such a situation, and that some direct estimation of eelworm population is required instead.

It was considered desirable to explore the effects on the host plant of both steam sterilization and D-D injection, both in the presence and in the absence of eelworm infection. It was therefore decided to set up a pot experiment in factorial form, making use of the three factors: partial steam-sterilization of soil (symbolized S), infection of soil with Heterodera cysts (H), and injection of soil with D-D (symbolized D), applied in that order. Each factor was applied to half the pots in such a way that all the eight possible combinations were equally represented: O, H, S, D, HS, HD, SD, and HSD. Since 40 large pots were available a 5-fold replication of each of the eight treatments was possible.

There is an anomalous feature about this design since, if D-D should kill all or most of the eelworms in any pot where both factors occur together (HD and HSD), the H factor would in fact be absent from

such pots. After due consideration it was decided to proceed nevertheless and to rely on analysis to solve this problem.

Apart from the primary intention of investigating the effects of these three factors on the host plants, the 20 infected pots (H) lent themselves to a secondary enquiry into the effects of factors S and D on the eelworm population. The primary intention requires a selection of suitable criteria for measuring the effects on the plants.

The present paper sets out the methods used in carrying out the experiment and the way in which suitable criteria were selected, leaving it to a second paper to discuss the results obtained. In the event, these results were considered sufficiently interesting to justify keeping the pots of soil and repeating the experiment, without renewing the treatments, in a second year. These second-year results will be discussed in a third paper.

Methods.

Soil. There was available a heap of good quality top-spit meadow soil, a medium loam, used for general potting purposes. This was found free from *H. rostochiensis* and was passed through a coarse sieve and divided into two equal portions, one of which was sterilized.

Sterilization was carried out in a small portable apparatus of the low-pressure type. Steam was passed through the soil until it reached a temperature of about 85° C. when it was allowed to cool and spread on a cemented floor to dry. Both this and the unsterilized soil were separately mixed with clean silver sand in the proportion by volume 14 : 5.

Pots were 12″ high and 13″ in diameter, and held 15 Kg. of soil to within 2″ of the rim. They had been coated with paraffin wax internally for a previous purpose.

Cysts of Heterodera rostochiensis were taken from a clean stock of about 20 gm. which had been previously floated out from an experimental soil. 20 lots of 800.0 mg. were weighed out for the experiment, and also 5 lots of 100.0 mg. for counting. The counts showed that 800 mg. would contain 23,800 cysts, with a standard error of 440 cysts. Dissection of 50 cysts showed that the mean number of viable eggs and larvae was 122.7 per cyst, or 2.92×10^6 per 800 mg. 52% of cysts were empty.

Infection. Half the sterilized and half the unsterilized pots were taken at random for infection. A potful of soil was spread on a clean cemented floor; the 800 mg. of cysts was thoroughly mixed with 50 ml.

of dry silver sand and the mixture evenly sprinkled over the soil, which was mixed by a shovel by coning and quartering and then replaced in the pot.

Injection of D-D into half the pots took place on the same date as infection (2/4/46). 10 ml. of D-D was run through a vertical glass tube plunged 6″ deep in the centre of each pot, after which all pots were lightly watered to form a seal. Watering was repeated on the second and third days since hot sun was cracking the soil surface. In so far as the area of soil surface can justifiably be compared with field areas, this rate of injection was equivalent to about 122 gallons/acre (1460 lb./acre). This high rate was selected because of the inconclusive results in field experiments at considerably lower rates.

Potatoes. 40 tubers of fairly even size, averaging 48 gm., were selected from a stock of 28 lb. Class A "Duke of York" Scotch seed, and lightly scrubbed with a soft brush with water to remove soil particles and cysts (if any). The sieved residue yielded two empty cysts of *H. rostochiensis*. The seed were chitted for 28 days when all eyes but two were rubbed out, and planted 2″ deep in the pots on the 28th day after injection (30/4/46). The pots were stood in four rows of 10 on a cement floor in an open but sheltered courtyard, and were randomized for position without restriction.

Criteria.

The effects of treatment on the growth of the potato plants were measured by a variety of criteria, namely : (1) Height of highest shoot, to the nearest centimetre, at 29, 45, 59, and 78 days after planting. This was measured by means of a horizontal bar sliding over a vertical rod calibrated in centimetres.

(2) Colour of foliage at 45 days after planting. Scores of −1, 0 and +1 were allotted respectively to plants showing light, medium, or dark green foliage. It was a matter of some surprise that an ordinary analysis of variance applied to these scores gave significant and sensible results.

(3) After the last height reading (17/7/46), when the foliage was yellowing and beginning to droop, the haulms were cut off level with the soil and oven-dried in linen bags at 105° C. to constant weight. The weight in grammes was used as a criterion.

(4) and (5) The number and weight of tubers from each pot were recorded, separating ware-with-seed from chats. The number of ware-with-seed tubers and the total number gave two items for the fourth criterion, and the corresponding two weights gave the fifth.

(6) In addition to the above plant criteria, this and the following were used for assessing the effects on the eelworm population of the two factors S and D, using only the 20 infected pots. Duplicate 200 gm. soil samples were taken from the air-dried and thoroughly mixed soil of each pot. These samples were washed in Fenwick's apparatus and the contained cysts floated up, collected and counted.

(7) Duplicate batches of 100 cysts from each soil sample were treated with calcium hypochlorite (Fenwick's method) and each batch made up to 50 ml. with water. In each case duplicate 1 ml. aliquots were withdrawn by pipette from the stirred volume, and the contained eelworm larvae counted. Thus, the total count for each pot represents in effect "larvae/8 cysts". The eggs failing to hatch in the hypochlorite were not counted.

Analysis.

The variance between the eight treatment means was expressed as a ratio to the error variance occurring within batches of 5 replicates, in the usual way, and only where this ratio (F) was significant were the treatments separately assessed. It should perhaps be explained that the data from all 40 pots are used for assessing not only each of the three factors (H, S, D) but also each of the interactions (HS, HD, SD, HSD. Thus, in the comparison :

$$(H + HS + HD + HSD) - (O + S + D + SD),$$

involving all pots, each term on the left differs only by the factor H from the corresponding term on the right, and the whole comparison is an unbiased estimate of the effect of the factor H.

As a basis for selecting suitable criteria, other things being equal, the higher the ratio (F), the more suitable the criterion. The value of the ratio depends partly on the degree to which the plant responds to treatment in respect of the criterion in question, and partly on the inherent variability of the criterion, which should be as low as possible. In the present case where criteria differ in the unit of measurement, the coefficient of variability or standard deviation in terms of the mean (V), is the appropriate statistic for assessing comparative variability.

Assuming a significant value for F in any given case, the average effect of each factor and each interaction can be assessed by taking into account the appropriate standard error. Thus, in the last paragraph but one, the difference between the sum of all the H pots and the sum of all the non-H will be judged significant if it sufficiently exceeds its standard error. The ratio of the difference to its standard error will be

Student's *t*, the distribution of which is known, and the critical value of which depends merely on the selected level of probability and the number of degrees of freedom used in determining the error variance. Such differences in terms of their standard errors will here be called **Normal differences.**

Other things being equal, the best criterion will be that which yields the most Normal differences large enough to be judged significant, or alternatively the largest Normal differences.

The results of the pot experiment can now be considered in the light of these three statistical measures, F, V, and *t*, and suitable criteria can then be selected for detailed analysis.

Before doing so there remains a problem, however, in that counts of cysts and larvae are not very suitable for analysis as they stand. Owing to the lethal effects of D-D, the analyses contain both high and low counts, and it is found that low counts are associated with low variability, and vice versa. For tests involving F and *t* it is essential that the mean and variance should be independent, and some kind of transformation is necessary.

Replicate counts of larvae approximate closely to the Poisson distribution, for which the appropriate transformation is to use the square root of the number counted. If the standard deviation, rather than the variance, is proportional to the mean, a logarithmic transformation is appropriate. Both have been used on the data of this experiment and the logarithmic one is preferred because, although slightly less accurate than the square root transformation, it is far more useful. The criterion "larvae gm. of soil" is obtained from the product of "cysts gm." and "larvae cyst" and, where logarithms are used, is simply got by adding the two values.

Results: Plant Criteria.

There are five plant criteria, but the first (Height of haulms) was determined on four occasions and the fourth and fifth have two items each, ware-with-seed and total), giving ten items in all. Analysis of variance applied to the results of each of these ten showed a significant value for F in each case, justifying a detailed examination of each of the seven components of the Treatments variance. These seven components are obtained from the various combinations of the eight treatment totals in contrasting groups of four, as illustrated in the "Analysis" section above for the factor H. The contrast yields a difference which, divided by its standard error, gives the Normal difference *t* for the factor in question. Under any given criterion, those Normal

differences will be judged significant which exceed the tabulated value of *t* for the selected level of probability.

Table I gives, under each of the ten items of the five criteria, (i) the *t*-values for each of the single factors and interactions, those significant at the 5% level being in bold type, (ii) the variance ratio, F, and (iii) the coefficient of variability, V. It should be pointed out that all the values in this table are pure numbers without dimensions, and the ten criterial items are comparable in terms of them. The criteria can now be discussed seriatim.

1. *Height of Haulms.*

At the 29th day after planting, the potato haulms vary considerably in height, partly because some are by chance late coming through. The coefficient of variability (V) is very high, and the variance ratio (F) is significant only because, even at this early stage, plants in the steam-sterilized soil have grown more rapidly than the others: S is the only factor with a significantly high *t*-value. From the aspects of low V and high F, the best day of the four is the 45th, later ones showing greater variability. The Day-45 results also show two single factors and two interactions to be significant, and the *t*-values are maximal for S (positive) and SD (negative); the only advantage shown by the data for the 59th day is that the *t*-values for D and DD (both positive) are maximal on that day.

The significant interaction DD occurs only with this criterion; it is significant on two of the four days and positive on all four, and is unlikely to be a chance effect. The other three significant components occur under other criteria.

2. *Colour of Haulms.*

It is interesting that this artificially scored criterion with, as expected, a very high variability, has yet provided Normal differences comparable with those for Height on the 59th day and altogether superior to (say) Weight of ware tubers. There is presumably a correlation between depth of green in the coloration of the haulms and their height. The criterion has no obvious advantages beyond ease of computation, and is in the nature of a curiosity.

3. *Dry Weight of Haulms.*

This criterion shares with "Weight of all tubers" an outstandingly high variance ratio and a moderate variability, together with closely comparable *t*-values. Of the two it is by far the more troublesome and time-consuming and there would seem to be no good reason for retaining it.

TABLE I.

Plant Criteria. Normal Differences (t) *for each Factor, also F and V.*
(Based on 20 values with, minus 20 without the factor.)

t-values in **bold** *type are significant.*

	(1) *Height of Haulms at day:*				(2) *Haulm Colour*	(3) *Dry Haulm*	(4) *Number of Tubers*		(5) *Weight of Tubers*	
	29	45	59	78	*Day 45*	*Weight*	*Ware*	*All*	*Ware*	*All*
[illegible]	1·38	0·08	0·18	1·02	0·30	0	—2·17	1·51	—1·88	—1·83
[illegible]	4·27	14·74	13·48	10·53	12·50	21·87	4·97	4·47	4·98	20·93
[illegible]	[illegible]	3·18	5·17	4·75	3·50	3·22	1·86	0·04	1·99	6·39
[illegible]	1·01	0·83	0·30	1·43	0·30	0·28	—0·16	0·18	—0·37	1·33
[illegible]	0·71	2·55	2·74	0·80	0·30	—0·08	0·47	1·58	0·34	0·82
[illegible]	[illegible]	4·04	5·30	[illegible]	2·80	4·25	—2·47	—2·43	—2·34	—4·74
[illegible]	[illegible]	1·58	[illegible]	[illegible]	[illegible]	2·16	1·24	2·50	1·00	—2·97
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	78·0	4·98	5·27	4·74	78·7
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	10·5	74·1	22·4	82·1	19·2

4. *Number of Tubers.*

In this case the interesting fact is that the number of all potatoes is more sensitive than number of ware-with-seed, having a lower V, higher F, and three as against two significant *t*-values. The only notable feature of the ware-with-seed analysis is that of all criteria it provides the only significant Normal difference for factor H : there are fewer ware potatoes in the infected pots.

5. *Weight of Tubers.*

This criterion resembles the last in that the weight of all potatoes is more sensitive than that of ware-with-seed, only in this case the contrast between the two is far more marked. Weight of all potatoes also is more sensitive than number of all; it is readily obtained, and in every way recommends itself as a valuable criterion.

General.

It will be seen that each of the ten criterial items shows a significant positive S effect. Six of them show a significant positive D effect and (not the same six) a negative SD interaction. The heights at 45 and 59 days show a significant positive HD interaction, and the three criteria : dry weight of haulms, number, and weight of all potatoes, a significant negative triple interaction.

It is likely that the experimental results can be fully explored by selecting for detailed consideration the two criteria : height at 45 days, and weight of all tubers. The only significant effect missed by these two is the negative H effect given by number of ware tubers, and even here the Normal difference for weight of all tubers approaches significance.

Results : Eelworm Criteria.

The 20 pots containing Heterodera cysts are available for the secondary purpose of investigating the effects of factors S and D, and their interaction SD, on the eelworm population as measured by cyst- and larva-counts. From this aspect, eelworm infection has ceased to be a factor in the experiment (H), and has rather taken the place of the plants as the experimental material under observation.

Table II is similar in construction to Table I and gives *t*-values (Normal differences) and also F and V for each of the eelworm criteria. Cyst counts are not suitable for measuring eelworm population, especially in fumigation experiments, because cysts remain in the soil long after their contents have escaped or been killed. Similarly, larvae

TABLE II.

Eelworm Criteria. Normal Differences (t) *for each Factor, also F and V.*
(Based on 10 *values with, minus* 10 *without the factor.)*

t-*Values in* **bold** *type are significant.*

Factor	*Cysts/gm. soil (c)*		*Larvae/Cyst (l)*			*Larvae/gm. soil (g)*	
	c.	*log c.*	*l.*	*(l) 1/2*	*log l.*	*g.*	*log. g.*
S	1·52	1·65	**2·71**	**2·47**	0·63	**2·18**	0·89
D	**—5·96**	**—5·92**	**—15·0**	**—21·7**	**—11·7**	**—10·4**	**—12·1**
SD	0·76	1·23	**—2·58**	—2·03	0·41	—2·10	—0·17
F	12·8	13·1	79·4	161	40·2	39·4	49·4
V%	14·4	2·20	28·5	15·4	18·3	41·5	26·1

per cyst is a unit which does not directly measure the eelworm population of the soil. The product of these two, larvae per gm. of soil, does give a satisfactory unit, however, provided that one has counted all the living larvae in the sample and no dead ones.

The purpose of Table II is not so much to provide a basis for selecting suitable criteria as to present the statistics of these eelworm distributions and to show the effects of transformations. It will be seen that both cysts/gm. and larvae/cyst (and therefore larvae/gm.) are characterized by high variability before transformation.

TABLE III.

Statistics for Two Distributions of unit : "Larvae/8 Cysts."

	A. D-D Absent.	*B. D-D Present*
Mean Squares : (D.F.)		
Treatments (1)	36906	21·025
Pots/Treatment (8)	5246	33·963
Batches/Pot (10)	6427	12·875
Aliquots (20)	567·15	4·075
N.	40	40
Mean ($\bar{x}$)	176·3	4·225
Variance(s^2)	567·2	4·075
Standard Dev. (s)	23·81	2·019
$s^2/\bar{x}$	3·22	0·96
$s/\bar{x}$	0·135	0·478

Table III gives the relevant statistics from a detailed analysis of two sets of counts with the unit : "larvae/8 cysts" ; each set comprises 40 counts (duplicate counts from each of two batches of 100 cysts per pot ; 10 pots without and 10 with D-D). The data have been separated into these two sets in order to show the relation between the mean and the variability in the extreme cases of high and low counts. It will be seen that the mean varies directly neither with the variance nor with the standard deviation, but somewhere between the two. On this evidence it is not possible to choose between the square-root and the logarithmic transformations, and the latter was in fact chosen because of its usefulness on other grounds. Thus, when working with logarithmic transformations (using the terminology of Table II) log g is the sum of log c and log l ; treatment means when re-transformed become geometric means, and the treatment differences ascribable to factors become ratios.

Summary and Conclusions.

1. Methods are described whereby a factorial experiment was set up to test the effects on 40 potato plants, growing in 15 Kg. pots of soil, of the three factors: (S) steam sterilization of soil, (H) infection of soil with about three million eggs and larvae of *Heterodera rostochiensis* per pot, and (D) injection of soil with 10 ml. of D-D per pot.

2. Data from the infected half of the pots were also used to test the effects of factors (S) and (D) on the eelworm population.

3. The data from 10 plant criteria yielded three statistics: the variance ratio (F), the coefficient of variability (V), and the Normal difference (t) corresponding to each factor and interaction. From an examination of these, the following two criteria are selected as, between them, displaying all the significant treatment effects of the experiment:

(a) Height of tallest shoot at 45 days after planting;

(b) Weight of all tubers produced.

4. A consideration of the eelworm counts gives grounds for using a logarithmic transformation of these counts for statistical analysis.

Acknowledgments.

The writer wishes to thank his colleague, Mr. D. W. Fenwick, for his full assistance in setting up the experiment, and for supervising and taking an active part in the eelworm counts.

Journal of Helminthology, Vol. XXII, Nos. 3/4, 1948. pp 128-138.

Potato Root Eelworm, D-D, and Soil Sterilization. II. Results for 1946.

By B. G. Peters, M.Sc., Ph.D.

(*Nematology Department, Rothamsted Experimental Station, Harpenden*).

An experiment, using potatoes in pots of soil, was set up in factorial form to investigate the effects on the growth of the plant of the three factors: (S) steam sterilization of soil, (H) infection of soil with about three million eggs and larvae of *Heterodera rostochiensis* per pot, and (D) injection of soil with D-D at the rate of 10 ml. per pot, the factors being applied in that order. Each factor was applied to half the pots in such a way that all the eight possible combinations were equally represented: O, H, S, D, HS, HD, SD, and HSD, and each treatment was replicated five-fold, making 40 pots in all.

In a previous paper (Peters, 1948) the methods have been set out in detail, and the results have been used for the purpose of selecting from 10 criteria applied to the plants two which, between them, brought out all the significant effects of the experiment. The two selected criteria were (i) Height in cm. of the tallest shoot at 45 days after planting, and (ii) Weight in $\frac{1}{4}$ oz. of all tubers produced; these two criteria are here examined in detail and the implications of the results are discussed.

The twenty pots receiving eelworm infection were also used for the secondary purpose of testing the effect of factors S and D, and their interaction SD, on the eelworm population as measured by cysts per gramme of soil (*c*), eelworm larvae per cyst (*l*), and their product: larvae per gramme of soil (*g*). Logarithmic transformations of the actual cyst- and larva-counts were used, taking log *g* for each pot as the sum of log *c* and log *l*.

To avoid possible confusion, throughout this paper Fisher's symbolism will be used to distinguish between treatments and factors. Thus, lower case initials in parenthesis will be used for treatments or the mean value of a criterion for the given treatment, and capital initials for the factors evaluated from all treatments. *E.g.* (hsd) is the treatment combining all three factors and represented by five replicate pots, while HSD is the triple interaction evaluated from the 40 pots as follows:

$$HSD = (h + s + d + hsd) - (hs + hd + sd + O).$$

Plant Criteria.

Table I shows, for each criterion, the mean value for each of the eight treatments, together with the general mean and the standard deviation. It will be seen even from these raw data that all the treatments containing (s) give higher values than the remainder, by both criteria.

Table I.

Summary of Treatments : Means per plant.
(Based on 5 plants per treatment

Treatment	*Height of Haulms at Day 45. (cm.)*	*Weight of all Tubers. (¼ oz.)*
Control	22·8	29·0
(h)	19·8	18·0
(s)	33·8	62·8
(d)	25·2	40·6
(hs)	33·4	65·4
(hd)	27·0	41·6
(sd)	32·2	68·8
(hsd)	33·6	64·6
Mean :	28·5	48·8
s :	2·05	5.00

A true measure of the effects of the factor S, however, can be obtained from the eight means by subtracting the sum of the remainder from the sum of the four containing (s). The difference will be in the unit "per four plants" and one quarter of this will give the difference per plant due to the effects of S :

$$S = (s + hs + sd + hsd) - (O + h + d + hd).$$

And similarly for the other two factors and the four interactions. The results of this process are shown in Table II, along with the standard error of such differences. Those differences which exceed *t*-times the standard error have been taken as significant. The various factors will now be considered one by one.

Eelworm Infection: H.

As Table II shows, this factor was negative but not significant under both criteria; in spite of a potential infection represented by three million eggs and larvae per pot, or about 200 per gm., there has been no significant effect on either height of plants or weight of tubers. As mentioned in the previous paper, there was a just significant effect

of eelworm under the criterion "No. of ware-with-seed tubers", but this result had a 3.8% chance of being a random effect, and other criteria gave non-significant results with positive and negative signs indifferently. The eelworm effect would therefore seem to be negligible.

The factor H is estimated from the comparison :

$$H = (h + hs + hd + hsd) - (O + s + d + sd).$$

If it should happen that D-D kills all eelworms, then the third and fourth treatments in the first component, (hd) and (hsd), are in effect reduced to the corresponding terms of the second component, (d) and (sd). Regarding these as cancelling out, the comparison reduces to :

$$H = (h + hs) - (O + s).$$

In other words, the comparison is still an unbiased estimate of H, but has only half its former strength.

This last expression also represents a comparison based on only half of the pots, namely on that half which excludes factor D. The value for factor H in Table II can be regarded as the resultant of a value for the 20 pots excluding D and the 20 including D. Alternatively, it can be analysed into the values for the half excluding and the half including S. This is done in Table III where the first pair of numerical columns gives the effects of factor H, respectively including and excluding the factors S and D, for each criterion in turn. The "Total" lines in the table are the resultants of either pair of values, and one-twentieth of the total value is the factor value of Table II.

It will be seen from Table III that the effect of H on height of haulms is negative in the absence of factor S, and still more so in the absence of D, but in each case positive in the presence of those factors. None of these effects is significant, but they are suggestive of a slight harmful effect of eelworm in the absence of the other factors. In the case of weight of tubers H is negative in the presence or absence of the other factors, but again the negative effect is more marked in their absence, and indeed is just significant in that half of the pots excluding S. It seems reasonable to suppose that the harmful effect of eelworm is offset in sterilized soil by the plants receiving a good start, but it would be very odd if the soil amendment effect were *better* in the presence of eelworm.

Soil Sterilization: S.

As Table II shows, the significant positive effect of S is by far the most pronounced response in the whole analysis. Consideration of the assumption that D-D kills all eelworms shows that this fact would not introduce any bias into the comparison, nor any dilution of the effect.

An examination of Table III shows that the S effects are all positive, and significantly so, whether the other two factors are present or absent. But the S effect is stronger in the presence of H, and in the absence of D, under both criteria.

TABLE II.

Effect of Factors : Mean Differences per Plant.
(Based on 20 plants with, minus 20 without the factor)
Values in **bold** *type are significant.*

Factor.	*Height of Haulms at Day 45. (cm.)*	*Weight of all Tubers. (¼ oz.)*
H	—0·1	—2·9
S	**9·6**	**33·1**
D	**2·1**	**10·1**
HS	0·6	2·1
HD	**1·7**	1·3
SD	**—2·8**	**—7·5**
HSD	—0·8	**—4·7**
S.E. (Difference)	0·648	1·58

TABLE III.

Analysis of Single Factors.
(Actual differences due to Factor (i) *in presence and absence of Factor* (ii).
Values in **bold** *type are significant.)*

	Factor (i)	*H*		*S*		*D*	
Criterion	*Factor* (ii)	+	—	+	—	+	—
Height of Haulms at Day 45. (cm.)	H	—	—	**101**	**90**	**37**	**4**
	S	5	—6	—	—	—7	**48**
	D	16	—17	**68**	**123**	—	—
	TOTAL	—1		**191**		**41**	
Weight of all Tubers (¼ oz.)	H	—	—	**352**	**310**	**114**	**88**
	S	—8	—50	—	—	26	**176**
	D	—16	—42	**256**	**406**	—	—
	TOTAL	—58		**662**		**202**	

The increased positive effect of S in the presence of H is linked with the decreased negative effect of H in the presence of S, noted in the previous paragraph. The situation suggests an interaction between these factors. Table II shows, however, that there is no significant HS

interaction, and one is driven to suppose that the situation is a random one. Even if it were not, it would be very difficult to find a sensible explanation of it.

The other anomaly, that the S effect is stronger in the absence than in the presence of D, is associated with a significant negative interaction, SD, under both criteria. Thus, there is good reason to believe that steam sterilization and D-D injection interfere with each other in some way: neither the height of haulms nor the weight of tubers is as great, in pots containing both factors, as would be expected if the factors acted independently. It might be said that sterilization, which was applied first, achieved a maximum effect (in releasing nitrogen, or whatever action it is supposed to have) so that the addition of D-D could not increase this effect; but this could not explain why the S effect is *less* in the presence of D-D.

D-D Injection: D.

The factor D has a significant positive effect under both criteria; in the case of weight of tubers, it is rather less than one-third the magnitude of the S effect. At this stage the interesting point arises whether D-D achieves its good effect through killing eelworms, or in some other way. Table III shows that in the absence of eelworm D-D has a negligible effect on height, but in its presence a significant positive effect. This suggests that the effect on height is achieved through killing eelworms; it is true that the H effect was not significant, but this may have been due to the diluting effect of the (hd) and (hsd) pots referred to above. Unfortunately, the assumed lethal effect of D-D would have the result of introducing a bias into the comparison upon which D is based. In the component "H absent" there is of course no bias, but it is doubly present in the "H present" component. Moreover, assuming H to have a negative effect, the bias works in each case in the direction of making the D effect positive. In these circumstances the only really trustworthy conclusion is that D-D has a negligible effect on height in the absence of infection. In the case of weight of tubers, there is a significant positive effect of D-D in the absence of infection, and this also can be accepted as trustworthy. This, in fact, is the soil amendment effect, isolated from any complications due to the presence of eelworm. The status of the other significant positive effects of D-D, whether in the presence of eelworm or generally over the 40 pots, is that they are likely to have been over-estimated to some unknown degree.

In the presence of S the effect of D is negative for height and positive

for weight but significant for neither. In the absence of S the effect of D is significant and positive under both criteria. Just as S was less effective in the presence of D, so is D in the presence of S. If D-D is lethal, both components suffer a slight bias towards the positive, but this situation can be regarded as the inverse of that discussed under S, and the expressions for S and its components, and SD, are unbiased in that the treatments (hd) and (hsd) come on opposite sides of the comparisons involved.

Interactions.

The interactions HS and SD have been sufficiently discussed; there remain HD and HSD, the former significant and positive under the height criterion and the latter significant and negative under the weight criterion. In the first place, if D-D kills all eelworms the factors H and D cannot coexist and the interactions HD and HSD are practically meaningless. The expression for HD is as follows:

$$HD = (hd + hsd + O + s) - (d + sd + h + hs).$$

A full lethal effect would reduce the first two terms of the left-hand group to the same as those of the second group. Cancelling these out, the expression becomes:

$$(O + s) - (h + hs),$$

and this is an inverted measure of the effect of H. In other words, a positive HD would be merely a reflection of a negative H. Without assuming a lethal effect the positive interaction might mean that H fails to exert its negative effect independently in the presence of D, which strongly points in the same direction.

In the same way, the expression for HSD reduces to an inverted estimation of HS, and a negative HSD would merely reflect a positive HS. HS is not significant under weight of tubers, but at least it is of opposite sign. The negative HSD interaction might therefore be taken as indirect evidence for the hypothesis that the positive effect of S is stronger, or the negative effect of H is weaker (or both), when the two factors occur together.

General.

This discussion has clearly brought out the complications which follow in a factorial experiment when one of the factors (D, in this case) is potentially able to neutralize the effects of another (H). Nevertheless, certain points have been well established and can here be summarized.

The factor H has not been shown to have any effect. The value which estimates it in Table II may have been diluted because of the (hd) combination, and the significant positive HD interaction under "height"

may for the same reason reflect a negative H, but these are no more than suggestive pointers.

The factor S has a very large positive effect which is slightly diminished in the presence of D. The large positive effect of steam sterilization on a soil which received no fertilizers was fully expected; the negative interaction with D was not expected and is difficult to explain.

The factor D has been shown to have a significant positive effect and, while the value of this factor generally, and in the presence of H, may have become inflated by the (hd) combination, its value in the absence of H is unbiased. The comparison of S with D in that half of the pots which contained no eelworm will give a relative measure of the soil-amendment effect of D-D injection. Table III shows that D-D has had no direct influence on the height of plants at 45 days, but in the case of the weight of all tubers it has significantly increased the yield, the ratio of the S effect to the D effect (310/88) being 3.5. The other way up, the D effect is about 28% of the S effect in the absence of eelworm.

Most of the interactions are suspect, through bias or dilution from the (hd) combination, but this does not apply to the negative and highly significant SD interaction. The mutual interference of sterilization and D-D injection in their effects on height and weight must be accepted as a fact. In the original selection of criteria, this interaction was negative for nine of the ten criteria tested and was significant for six of them.

Eelworm Criteria.

Table IV shows the geometric mean counts of cysts and larvae for each of the four treatments involving eelworm, together with the general mean and the coefficient of variability. The standard deviation is in logarithmic form and has no clear meaning if re-transformed; in relative form it at least shows the greater variability of larvae per cyst compared with cysts per gm. of soil.

The original addition of 23,800 cysts to each pot corresponds to a density of about 1.59 cysts/gm. Since about 52% of original cysts were empty, the density of viable cysts was about 0.826 cysts/gm., and that of empty cysts about 0.764 cysts/gm. In Table IV the mean of the two treatments lacking D-D, less the originally empty cysts, gives 1.70 viable cysts/gm., or 2.06 times original density. Similarly, the

mean of the two treatments including D-D gives 0.916 viable cysts/gm., or 1.11 times the original density. Thus, in the absence of D-D the viable-cyst density has doubled, and in its presence has not appreciably changed.

Corrections for originally empty cysts, applied to the logarithmic data in analysis, lead to larger treatment differences but, since the standard error is increased in about the same proportion, no advantage results.

TABLE IV.

Geometric Mean Counts of Eelworms.
(*Based on 5 pots per treatment*)

Treatment	*Cysts/gm. of soil* (*c*)	*Larvae/cyst* (*l*)	*Larvae/gm. of soil* (*g*)
(h)	2·43	71·3	171
(hs)	2·50	102	252
(hd)	1·52	1·44	2·17
(hsd)	1·84	1·55	2·82
Mean :	2·03	11·3	22·7
V :	2·20%	18·3%	26·2%

In the case of larvae per cyst, eggs and larvae were originally determined by dissection, and not by the use of the hypochlorite technique; no direct comparison of before and after is therefore possible. It should also be pointed out that the larval counts can not be taken as measures of kill by D-D. Whatever the original kill, the counts given include larvae from the new cysts formed on the potatoes during the experiment.

Larvae per gm. of soil were obtained by adding the logarithms of cysts/gm. and larvae/cyst. In Table IV they are the product of the first two, allowing for errors of approximation in re-transformation.

In logarithmic transformation the effect of factors can be estimated from all twenty infested pots, in the same way that Table II was derived from Table I. When re-transformed, these differences due to factors become ratios as shown in Table V. In the case of ratios less than unity, the reciprocal is also shown as making comparison with the others easier: such ratios correspond to negative effects in logarithmic form.

The only significant effect is the reduction due to factor D, which is very great in terms of larvae per gm. : there are 84 times as many larvae in the uninjected as in the injected pots; in other words the latter contain only 1.18% of the population of the former. Something approaching a 99% reduction shows that the factor H has been effectively eliminated from all pots in which it was present along with factor D, and that this complication mentioned in discussing plant criteria has indeed existed.

TABLE V.

Effect of Factors : Eelworm Ratios.
(Ratios of 10 pots with, to 10 without the factor)
Values in **bold** *type are significant.*

Factor	(*c*)	(*l*)	(*g*)
S	1·11	1·24	1·38
D	**0·678 (1·48)**	**0·0175 (57·0)**	**0·0119 (84·0)**
SD	1·08	0·910 (1·10)	0·940 (1·06)

The other effects are not significant as they stand. Table II of the previous paper in this series, however, shows that S was significant when using a square-root transformation, and SD almost so. It is therefore legitimate to consider them as possibly real effects. The positive S effect, giving 1.38 times as many larvae in the sterilized as in the unsterilized pots, would not be unexpected. Steam sterilization led to larger and better plants which would be able to support a larger population of new cysts than the others. A negative SD interaction was found in the plant criteria, and there is some sign of it here also. It is difficult to believe that the lethal effects of D-D are greater in steam-sterilized soil; but easier to regard it in this case, if real, as a reflection of the plant situation. If the beneficial effects of S on the plants are reduced by the presence of D, such plants will show less of the positive S effect just mentioned, and will support fewer eelworms than the (s) plants not so affected.

Summary and Conclusions.

1. A factorial experiment involving the presence and absence of three factors: steam sterilization (S), infection with some three million eggs and larvae of *Heterodera rostochiensis* per pot (H), and injection of 10 ml. of D-D per pot (D), the factors applied in that order, was carried out to ascertain the effects on the growth of 40 potato plants in pots containing 15 Kg. of soil.

2. In the event, the D-D was lethal to the great majority of eelworms, so that treatments labelled (hd) and (hsd) were effectively reduced to (d) and (sd). This fact greatly complicated the analysis, but nevertheless enabled certain conclusions to be drawn. The use of this factorial design may be criticised in the face of this antagonistic action between two of the factors. Nevertheless, the effects of H and D acting together on the plants was a subject of interest; the real fault in design lay in using too high a concentration of D-D, or in not using a series of concentrations. The fact that the concentration used was too high emerged from the experiment and was not known beforehand.

3. It has not been shown that eelworm infection has had any significant effect on the plants, except possibly on the weight of tubers in the absence of soil sterilization. This applies to the pots not receiving D-D, as well as to the others, and is therefore not merely a result of the antagonistic-factor situation.

4. As expected, soil sterilization has a highly significant positive effect on both height and weight (and, indeed, on the other eight criteria originally tested). The effects are somewhat reduced in the presence of D-D, and this result is reflected in a significant negative interaction SD. This is tentatively explained by assuming that soil sterilization has a maximum effect which therefore cannot be increased by D-D.

5. Although the general positive effects of D-D are suspect, owing to antagonism between D and H, the unexceptionable results in the absence of eelworm show that D-D has no significant effect on height of haulms, but leads to a significant increase in the yield of all tubers. This is a measure of the soil-amendment effect, and is about 28% of the corresponding steam-sterilization effect.

6. D-D leads to a highly significant reduction in the density of cysts and larvae of *Heterodera rostochiensis* as measured after the growth of an intervening host crop. The larval population in the D-D pots was only 1.18% of that in the others.

7. There are slight indications of a positive S effect and a negative SD effect in the larval counts. If real, these can probably be explained in terms of the corresponding effects on the host plants, assuming that the better the plant the higher the eelworm population (under the conditions of this experiment).

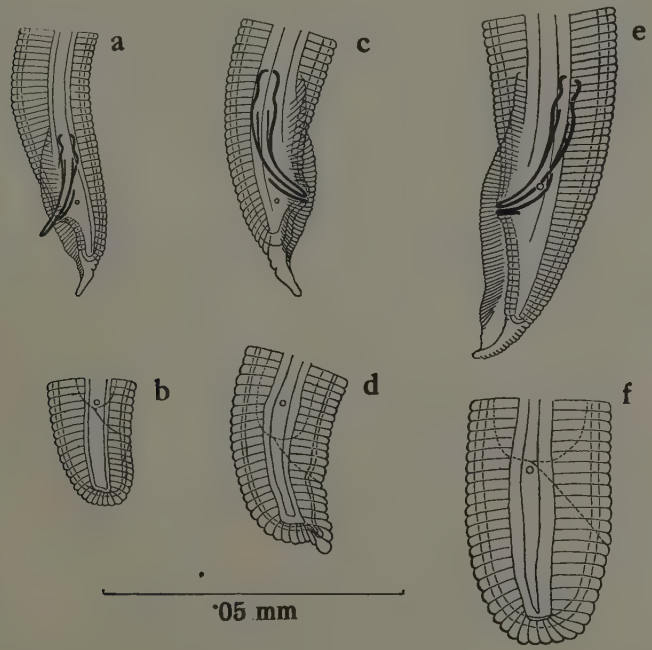
Journal of Helminthology, Vol. XXII, Nos. 3/4, 1948. pp. 139—140.

A note on the presence of phasmids on the male tails of *Anguillulina multicincta*, *A. erythrinae* and *A. robusta*.

By T. Goodey, D.Sc., F.R.S.

(*Department of Nematology, Rothamsted Experimental Station, Harpenden*).

In an earlier paper Goodey (1940) gave a detailed description of the morphology of three species of *Anguillulina* viz. : *A. multicincta* (Cobb) Goodey, *A. erythrinae* (Zimmermann) Goodey and *A. robusta* (de Man) Goodey. All three species are associated with plant roots : *A. multicincta* is a parasite of banana roots, *A. erythrinae* is parasitic on the roots of certain common grasses and cereals such as *Agrostis stolonifera, Lolium perenne* and oats (the writer has also found it on hop



Explanation of Figures.

Fig. 1. Lateral views of male and female tails, all drawn to the same magnification : a & b, *Anguillulina multicincta* (Cobb) Goodey ; c & d, *A. erythrinae* (Zimmermann) Goodey ; e & f, *A. robusta* (de Man) Goodey.

roots), *A. robusta* though associated with grass roots has not, to the writer's knowledge, been found within plant tissues. In the case of all three species phasmids were easily recognized on the female tails and were described and figured but they were not seen on the male tails and it was assumed that they were probably absent.

Further examinations which the writer has recently made show that phasmids are also present on the male tails of these three species. Careful examination of the tail in lateral aspect, under oil immersion and with critical illumination reveals a small bright spot situated within a slight depression in the expanded area of the lateral field in the anal region. In *A. multicincta* and *A. robusta* it is somewhat preanal in position whereas in *A. erythrinae* it is adanal. The papilla-like core of the phasmid is directed laterally and is not in any sense a ray supporting the caudal wing of cuticle. In the case of *A. erythrinae,* of which several males were available for study, a few were lying in dorso-ventral aspect and the phasmids were recognizable as a conical prominence arising, one on either side, from the body. In Fig. 1 the female tails of the three species as well as the male tails are represented so as to show the position of the phasmid in relation to the lateral field and the anus.

Reference.

Goodey, T., 1940.—"On *Anguillulina multicincta* (Cobb) and other species of *Anguillulina* associated with the roots of plants." *J. Helminth.*, **18** (1). 21–38. (*W.L.*11224b)

Journal of Helminthology, Vol. XXII Nos. 3/4, 1948. pp. 141—164.

*On some new species of Spirurids from Terrestrial Vertebrates, with notes on *Habronema mansioni*, *Physaloptera paradoxa* and *Hartertia zuluensis*.

By C. C. Kung, Ph.D., B.Sc.

Research Assistant in Zoology, Institute of Zoology, Nanking.

(From the Department of Parasitology, London School of Hygiene and Tropical Medicine.)

Collections of nematodes from two main sources provided the material for the following study on Spirurids of terrestrial vertebrates. Nematodes of this group have not infrequently been received in the Department of Parasitology, London School of Hygiene and Tropical Medicine, from Amphibians, Reptiles, Birds and Mammals dying at the Zoological Gardens, London, during the past twenty years and some of these were kindly placed at the disposal of the writer for examination. Three new species were found from Zoo animals which are described herein under the names : *Camallanus multilineatus* n.sp., *Tanqua gigantica* n.sp. and *Ancyracanthopsis madagascariensis* n.sp. A species of *Streptocara*, possibly new, is also described. The collection contained specimens of *Habronema mansioni* and *Physaloptera paradoxa* whose morphology is critically examined.

To Dr. P. Leroux the writer is much indebted for the privilege of examining some Spirurids collected by him in South Africa, which included two new species, *Camallanus mazabukae*, n.sp. and *Procamallanus brevis* n.sp. from bull-frogs, and *Physaloptera* sp. inq. from a mongoose. A single female *Camallanus*, probably a new species, and the hitherto unknown female of *Hartertia zuluensis* are described.

The Departmental collection also furnished a new *Physaloptera* from South America. The host and donor are unknown, on account of an indecipherable label, but on morphological grounds it is assumed that this species was harboured by a monkey.

Family: Camallanidae Railliet and Henry, 1915.

Genus: Camallanus Railliet and Henry, 1915.

Camallanus mazabukae n.sp. (Figs. 1-3.)

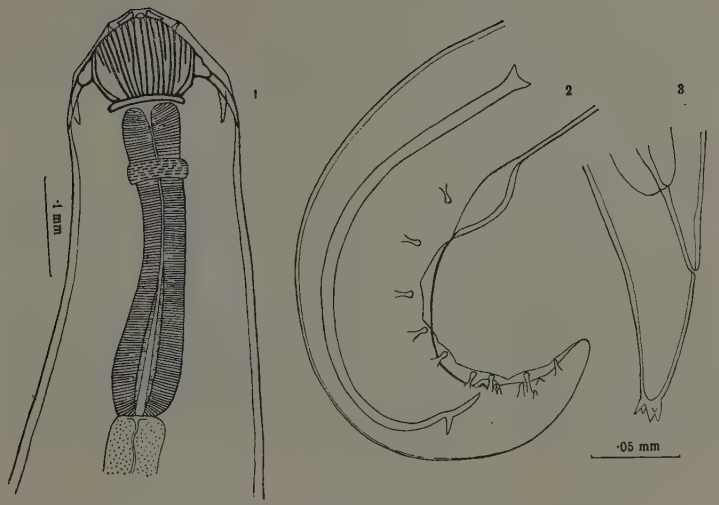
Two specimens (a female and a male) were obtained from the intestine of the bull-frog in South Africa.

*Part of a thesis approved by the University of London for the award of the Ph.D. Degree.

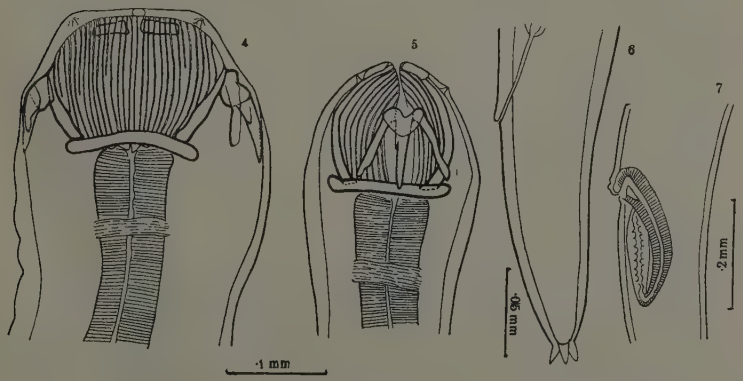
These small worms measure 3.47 and 2.51 mm. long by 0.27 and 0.20 mm. wide in the female and male respectively. The body tends to curve ventrally and is much diminished in thickness towards the posterior end, particularly in the female. Its cuticle is strongly striated transversely throughout the length of the body. The mouth is a slit-like opening with six small papillae, four submedian and two lateral in position. The lateral valves of the buccal capsule bear on each side sixteen to eighteen longitudinal ridges. In the female the buccal capsule is 0.08 mm. deep by 0.095 mm. wide in the middle and 0.057 mm. wide at the base; the corresponding dimensions in the male are 0.069 mm. deep and 0.075 mm. wide in the middle and 0.049 mm. wide at the base. Two quadrilateral cuticular thickenings are present on the anterior edge of the buccal valve. The lower part of the buccal capsule is thicker than the upper anterior part and its base is very much thickened to form a rim articulating with the oesophagus. The dorsal and ventral tridents each have three subequal prongs, the middle prong being 0.039 mm. long in the male; they extend behind the anterior end of the oesophagus. The nerve ring is situated at 0.14 and 0.11 mm. from the anterior end in the female and male respectively. The oesophagus is sharply divided into two more or less equal parts, the total length being 0.63 and 0.45 mm. long in the female and male respectively. The anterior muscular part is club-shaped posteriorly and the posterior glandular part is more or less uniform in thickness throughout its length.

The female: The conical tail, 0.075 mm. long, terminates in five cuticular spines, three of which are longer than the others. The vulva is situated just behind the middle of the body and is easily recognized by its thickened rim. The muscular walled vagina, 0.068 mm. long, runs backwards as well as dorsalwards and is connected in the posterior half with the two uteri. The uteri are fully distended with numerous larvae which are 0.44-0.49 mm. by 0.016 mm. wide.

The male: The tail is bluntly conical without digitations. The caudal alae are long and narrow but conspicuous, beginning at a cuticular swelling on the ventral side of the body at 0.615 mm. from the posterior end and extending down to the tip of the tail. There are ten pairs of lateral pedunculate papillae: six equidistant preanals, one adanal and three postanals of which the first two are grouped together while the third one is more isolated. Very close to the anus there are two or possibly three pairs of small circumanal papillae. Also on the ventral caudal surface there are one or two pairs of small papillae asym-



Camallanus mazabukae n.sp.
1. Lateral view of anterior end of body. 2. Lateral view of male tail. 3. Lateral view of female tail.
Scale .05 mm. refers to Figs. 2 and 3.



Camallanus sp.
4. Lateral view of anterior end of body. 5. Dorsal view of anterior end of body.
6. Lateral view of female tail. 7. Lateral view of female genitalia.
Scale .1 mm. refers to Figs. 4 and 5.

metrically arranged behind the second postanals. Only the right spicule can be distinctly seen. It is 0.315 mm. long with a lateral spine near its terminal end. No left spicule could be seen.

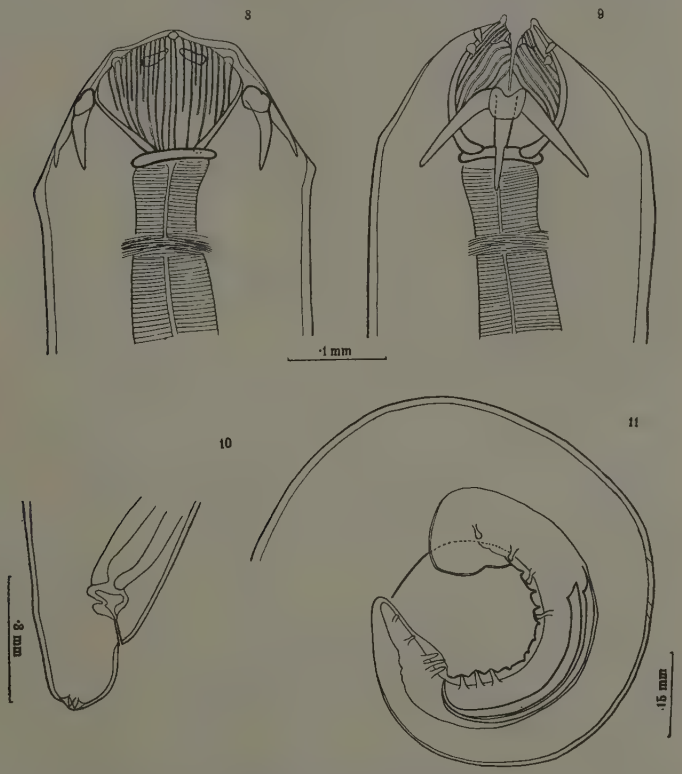
Walton (1935) gave a table showing five species of this genus from amphibians and their differences from one another, namely : *C. microcephalus* (Dujardin, 1845) Railliet and Henry, 1915, *C. nigrescens* (Linstow, 1906) Railliet and Henry, 1915, *C. baylisi* Karve, 1930, *C. multiruga* Walton, 1932 and *C. pipientis* Walton, 1935. Subsequently Southwell and Kirshner (1937) recorded another species, *C. kaapstaadi* from the clawed toad (*Xenopus laevis*) in South Africa. The present species is evidently allied to *C. kaapstaadi,* both of them being easily differentiated from the other five by their small body dimensions. But it differs from *C. kaapstaadi* in the smaller number of the buccal cuticular ridges, in the longer tridents, in the presence of two (three?) pairs of circumanal caudal papillae, in the right spicule having a lateral spine at its terminal end and in the non-digitation of the male tail.

Camallanus sp. inq. (Figs. 4-7.)

A single young female *Camallanus* measuring 2.72 mm. long by 0.184 mm. thick, was obtained from the intestine of the bull-frog in South Africa. The body diminishes in thickness posterior to the middle of the body and its cuticle is prominently striated transversely. The buccal capsule is 0.121 mm. deep by 0.172 mm. in maximum width and the lateral valves bear 29-32 longitudinal ridges on each inner surface. Around the mouth there are six cephalic papillae, four being submedian and two lateral in position. The tridents are about 0.079 mm. long and do not extend behind the anterior end of the oesophagus. The nerve ring and cervical papillae are situated at 0.20 and 0.32 mm. from the anterior end respectively. The length of the oesophagus is 0.86 mm. and that of its anterior muscular portion is 0.39 mm. The vulva, elevated from the cuticular surface, is covered by an anterior lip and is situated at 1.69 mm. from the anterior end. It leads into a rather short vagina about 0.248 mm. in length. The uterine larvae are not fully formed in this young specimen. The tail measures 0.135 mm. and terminates in three processes. This specimen is very similar to *C. kaapstaadi* Southwell and Kirshner, 1937, but in the absence of males it cannot be identified with certainty.

Camallanus multilineatus n.sp. (Figs. 8-11.)

A single female and two males were obtained from the stomach of *Rana catesbiana* from N. America. They are whitish, slender worms; the female and male measure 30.4 and 10.5-11.1 mm. long by 0.74 and



Camallanus multilineatus n.sp.
8. Lateral view of anterior end of body. 9. Dorsal view of anterior end of body. 10. Lateral view of female tail. 11. Lateral view of male tail.
Scale .1 mm. refers to Figs. 8 and 9.

0.37 mm. thick respectively. The body cuticle shows prominent transverse striations throughout its length. The mouth has six papillae (two lateral and four submedian) around its margin. The buccal valves are strongly chitinized with 22-24 longitudinal ridges on each side, some

of which are incomplete. There are at the anterior edge a pair of quadrilateral cuticular thickenings on each valve. The buccal capsule is broader than long, measuring 0.15 and 0.116 mm. long by 0.176 and 0.138 mm. in maximum width in the female and male respectively. The well-developed tridents extend behind the anterior end of the oesophagus and the three prongs are about equal in length, namely, 0.105 mm. The total oesophagus length is 1.46 and 1.17 mm. in the female and male respectively; its anterior club-shaped part is 0.628 and 0.45 mm. long in the female and male respectively. The nerve ring encircles the anterior part of the muscular oesophagus at 0.27 and 0.20 mm. from the anterior end in the female and male respectively. The cervical papillae are very minute, situated a little behind the nerve ring. The excretory pore was not seen.

The female: The tail, 0.15 mm. long, is rounded and bears three very small cuticular processes at the extremity. The vulva, situated 9.5 mm. from the anterior end, is without prominent lips and the uteri contain numerous fully-formed larvae.

The male: The tail is strongly curved ventrally, and bears narrow caudal alae about 0.75 mm. long. There are seven preanal papillae, the first four pairs being more or less equidistant from one another while the last three are grouped together near the anus. The adanal papillae are not visible on account of the strong curvature of the tail. There are five pairs of postanal papillae; the first three pairs are grouped together just behind the anus; the fourth pair is situated mid-way between the anus and the tip of the tail and the last is near the tip. The two spicules are subequal, the right one being 0.425 mm. long and nearly twice as wide as the left one which is about 0.4 mm. long. They appear to terminate in a simple point.

Of the seven species of this genus reported from Amphibians, the present species is distinguished from *C. kaapstaadi* and *C. mazabukae* by its larger dimensions. It differs from the remainder in the greater number of buccal ridges, in the different disposition of the caudal papillae and in the relatively anterior position of the vulva. It is still further removed from *C. baylisi* by having two subequal spicules of different shape and by the non-digitation of the male tail.

Family: CAMALLANIDAE Railliet and Henry, 1915.

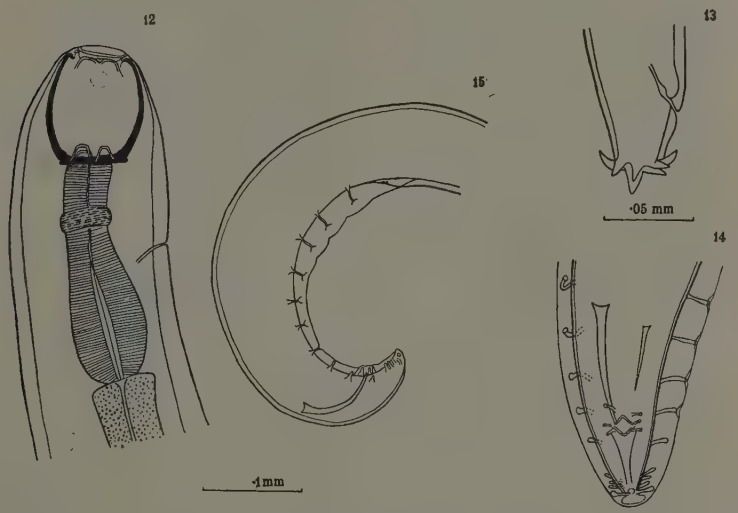
Genus: PROCAMALLANUS Baylis, 1923.

Procamallanus brevis n.sp. (Figs. 12-15.)

About a dozen female and male specimens were collected from the

intestine of the bull-frog in S. Africa.

The mature worms are small, measuring 1.95-2.1 mm. and 1.75-1.85 mm. long by 0.15-0.175 mm. and 0.083-.092 mm. wide in the female and male respectively. The body begins to attenuate at one-third of its length from the posterior end. It tends to curve ventrally and its cuticle is distinctly striated transversely. The terminal mouth opening



Procamallanus brevis n.sp.
12. Lateral view of anterior portion of body. 13. Lateral view of female tail.
14. Ventral view of male tail. 15. Lateral view of male tail.
Scale .1 mm. refers to Figs. 12 and 15.
Scale .05 mm. refers to Figs. 13 and 14.

is circular with six small papillae, four of which are submedian and two lateral in position. The barrel-shaped buccal capsule is continuous and appears to be laterally compressed. Its wall is highly chitinized without any ridges. It is about 0.11 mm. deep by 0.09 mm. in maximum width in the female; the corresponding dimensions are 0.09 and 0.065 mm. in the male. The lower part of the buccal wall is thicker than the upper anterior part and its posterior margin is greatly thickened to form a rim articulating with the anterior end of the

oesophagus. At the base of the buccal cavity there are three dome-shaped cuticular processes projecting into the cavity. In many specimens degenerated red blood cells of the frog were seen in the buccal cavity which suggests that they may be blood suckers. The anterior muscular part of the oesophagus is club-shaped, and measures 0.198-0.22 and 0.165-0.171 mm. long in the female and male respectively. The nerve ring is at .016 mm. from the anterior end of the female and the excretory pore is situated behind it; the corresponding parts of the male are 0.132 and 0.172 mm. respectively from the anterior end.

The female: The tail is 0.016 mm. long and terminates in 5-7 spinous processes. The vulva is situated behind the middle of the body, usually 0.83-0.904 mm. from the posterior end. It leads into the vagina which is 0.51 mm. long and runs back nearly to the tail region where it is connected with two uteri greatly distended with numerous larvae.

The male: The tail is blunt and coiled ventrally. The slender caudal alae are about 0.32 mm. long. There are eight or nine pairs of equidistant preanal papillae, one pair of adanal, three pairs of small circumanals and four pairs of postanals of which the final pair is the largest and arises more towards the mid-ventral line. Between this final pair there is an unpaired sessile papilla. The spicules are unequal, the right being 0.079-0.089 mm. long and the left, which is so poorly chitinized and slender that it is easily overlooked, is 0.039 mm. long.

Twenty-two species of this genus appear to have been recorded. With the exception of two species, viz. *P. xenopodis* Baylis, 1929 and *P. slomei* Southwell and Kirschner, 1937, which are from Amphibians, all are parasites of fishes. The species from Amphibians are characterized by two features :

(1) Short body length, not more than 3 mm. in the female as compared with those from fishes which vary from 5 to 33 mm. the majority being over 10 mm. long;

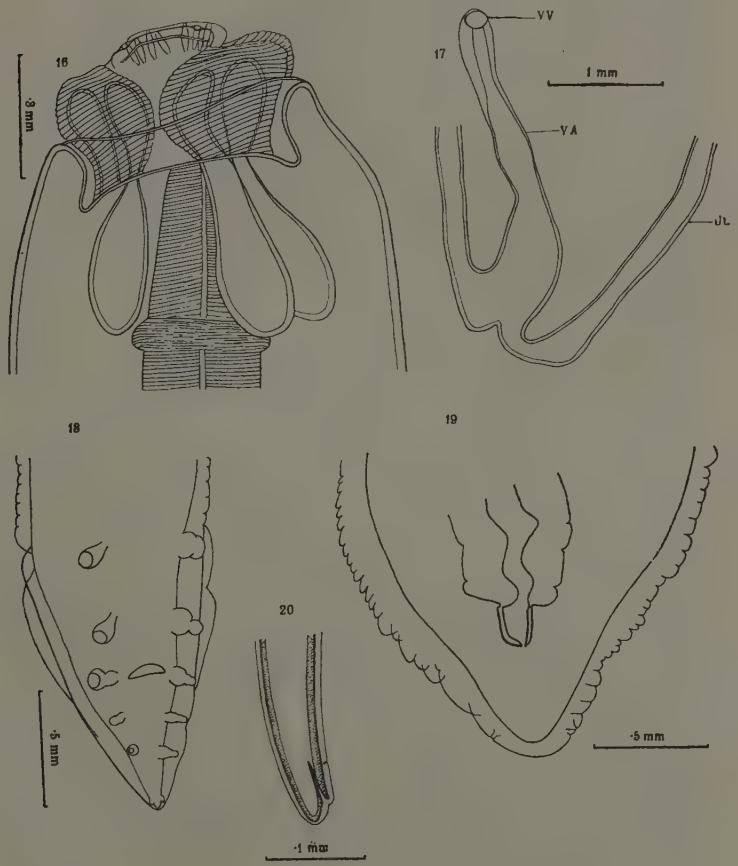
(2) The female tail terminates in at least five long spinous processes instead of a simple tip or 2-3 short blunt spines.

The new species is thus closely allied to *P. xenopodis* and *P. slomei,* but it lacks the striated buccal capsule of *P. xenopodis* and differs from *P. slomei* in the presence of three pairs of small circumanal papillae, the three additional ventral papillae at the tip of the tail and in the relatively posterior position of the vulva. As regards the male spicules it appears possible that the left spicule of *P. slomei* may have been overlooked.

Family: GNATHOSTOMIDAE Railliet, 1895.
Subfamily: GNATHOSTOMINAE, Baylis & Lane, 1920.
Genus: TANQUA Blanchard, 1904.

Tanqua gigantica n.sp. (Figs. 16-20.)

Two collections consisting of seven females and three males were



Tanqua gigantica n.sp.
16. Lateral view of anterior end of body. 17. Female genitalia. 18. Latero-ventral view of male tail. 19. Ventral view of female tail. 20. The terminal end of spicule. VV—Vulva, VA—Vagina, UT—Uterus.

obtained from the intestine of *Python reticulatus* and the oesophagus of *Naja hannah* in E. Indies and S.E. Asia respectively.

These worms are very large and stout, the female measuring 120-160 mm. in length and 2.5-3.2 mm. in thickness and the male 110-130 mm. and 2-2.5 mm. respectively. They are slightly attenuated towards the anterior end, and the body cuticle has very fine transverse striations throughout its length. The well-developed head bulb is divided by lateral grooves into two swellings situated dorsally and ventrally, about 0.57 mm. in transverse diameter, which are prominently striated. The cervical sacs are 0.47-0.50 mm. long. The two lateral lips are prominent, each of them bearing at its dorsal and ventral corners two submedian papillae, but lateral papillae were not seen. The oesophagus is slender, about 16.25-16.67 mm. long by 0.82-0.96 mm. wide at the posterior end in both sexes. The nerve ring is at 0.68 mm. and the minute cervical papillae 1.27 mm. from the anterior end in the female. The excretory pore could not be seen.

The female: The posterior extremity of the body ends rather abruptly in a short tail about 0.55 mm. long which bears a pair of papillae at about 0.22 mm. from the tip. The vulva is inconspicuous and situated at a distance of about 52.5 mm. from the posterior end. It opens into a muscular vagina which runs forward for a distance of 2.5 mm. and gives off two branches. They immediately turn sharply backwards, one going on to the posterior ovary and the other turning upon itself, running forward and leading into the anterior ovary. They are filled with thin-shelled, fully embryonated eggs which measure about 0.063 × 0.044 mm.

The male: The body is attenuated towards the posterior end and has narrow caudal alae. There are six pairs of bulky caudal papillae : two preanals, one adanal and three postanals, which diminish in size in the order named; the last pair is very small. The two spicules are equal in length and similar in build with blunt ends, and measure 1.45 mm. long by 0.06 mm. broad—about 1/90 of the body length.

Hitherto three species have been recorded from monitor lizards and snakes, namely : *T. tiara* (Linstow, 1879) Blanchard, 1904, *T. anomala* (Linstow, 1904) Baylis, 1916 and *T. diadema* Baylis, 1916. These vary from 20 to 60 mm. long and from 0.11 to 2 mm. thick. The present species is thus exceptionally long and stout. It is evident that the new species is allied to *T. anomala* and *T. diadema* on account of the similar head bulb and the same number of the uterine branches; but in addition to the difference in the body dimensions it is further distinguished from

them by the presence of six pairs of caudal papillae instead of eight and by the different ratio of the spicule length to the body length. *T. tiara* can be excluded by its head bulb with four swellings as well as four uterine branches.

Family: Ancyracanthidae Railliet, 1916.
Subfamily: Schistorophinae Travassos, 1918.
Genus: Ancyracanthopsis Diesing, 1861.
Ancyracanthopsis madagascariensis n.sp. (Figs 21-24.)

This material consists of five females and a single male collected from the gizzard of *Dryolimnas cuvieri* in Madagascar, Africa.

The body is filiform, translucent white with fine transverse striations throughout its length, and measures 14.44 and 6.14 mm. long by 0.27 and 0.12 mm. thick in the female and male respectively. It becomes gradually diminished in thickness from the glandular oesophagus forwards. The mouth is guarded by two lateral dome-shaped lips with a tooth-like projection at its summit; it bears six papillae, four submedian and two lateral in position. The head is armed with four cuticular appendages, about 0.031-0.048 mm. long, each of which is sub-divided into four posteriorly directed processes which vary from 0.013-0.022 mm. long; they are not pinnate. The vestibule is cylindrical, 0.037-0.04 mm. and 0.032 mm. long in the female and male respectively; its lumen is about 0.003-0.004 mm. in diameter at the base. The oesophagus consists of two portions, the anterior muscular one being 0.815-0.973 mm. long by 0.056 mm. wide and the posterior one 7.709-1.953 mm. long by 0.091-0.11 mm. wide in the female; the corresponding parts in the male are 0.81 and 1.157 mm. long by 0.059 and 0.078 mm. wide respectively. The cervical papillae are small and situated at about the level of the nerve ring which is at 0.15-0.18 mm. from the anterior end. The excretory pore is a little behind the nerve ring. No lateral body alae were seen.

The female: The tail narrows abruptly just behind the anus; it bears a pair of papillae at its tip and is 0.102-0.131 mm. long. The vulva is at 7.41 mm. from the anterior end. The vagina appears to be very long but actual measurements are impossible to give as it is obscured by the uteri. The embryonated eggs are 0.039-0.04 by 0.025-.028 mm.

The male: The posterior end is rolled in a loose spiral. The caudal alae are long and narrow, there are six pairs of preanal pedunculate

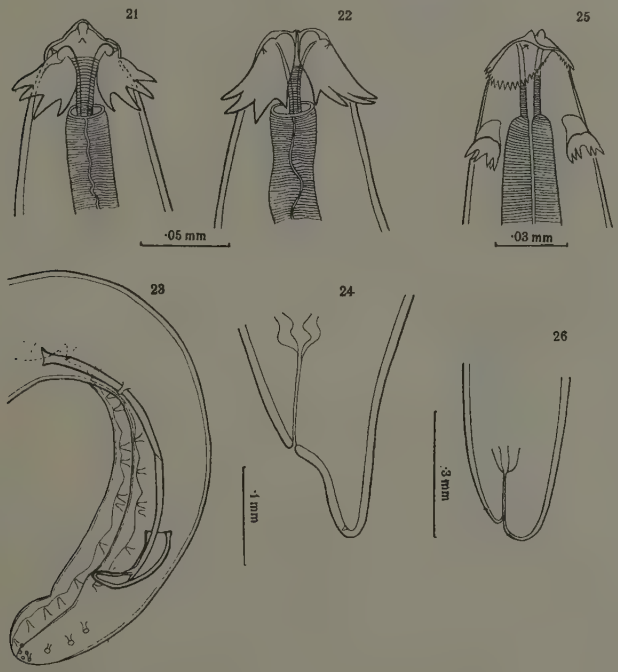
papillae on the left side and ten pairs on the right side. Posterior to the anus there are five pairs of lateral pedunculate papillae. A group of four small sessile papillae is situated ventrally at the tip of the tail. The two spicules are unequal and dissimilar, the right being 0.1 mm. long, very thick and navicular in shape, and the left being 0.3 mm. long, filiform and ending in 2-3 small points. No gubernaculum was seen.

In view of the similarity in the general pattern of the male caudal papillae and the spicules the present species appears to be allied to *A. bihamata* (Mueller, 1897) Cram, 1927, but it differs from it in the much longer spicules, in the asymmetrical arrangement of the caudal preanal papillae and in the situation of the vulva at the middle of the body instead of posterior to it. Furthermore, its head bears no crown of papillae or projections at the base of the large process of the cephalic appendages as described in *A. bihamata.*

Up to the present there are four species in this genus viz. *A. bilabiata* (Molin, 1860) Diesing, 1861, *A. bihamata* (Mueller, 1897) Cram, 1927, *A. fausti* Li, 1934 and *A. madagascariensis* n.sp. It is doubtful whether these all belong to the same genus. Firstly, there are considerable differences in the cephalic appendages. According to Molin (1860) and Diesing (1861) the type species *A. bilabiata,* has four pinnate posteriorly directed appendages but Drasche (1884) stated, and his figure shows, that each of the appendages subdivides into four equal digitations which are not pinnate. Describing *A. bihamata,* Müeller (1897) and Cram (1927) state that the head has four posteriorly directed pointed processes, 0.023-0.04 mm. long, similar to those of *A. bilabiata.* Yet as shown in Mueller's figure the processes appear to be undivided thus differing from those of *A. bilabiata.* In *A. fausti* the head is armed with only two posteriorly directed appendages though each of them subdivides into four subequal digitations. In the new species the cephalic appendages appear more or less similar to those of *A. bilabiata* because its head is furnished with four posteriorly directed appendages, each subdividing into four subequal digitations. Secondly, the position of the vulva is variable in the different species. It is posterior to the middle of the body in *A. bilabiata* and *A. bihamata* and at the anterior sixth of the body in *A. fausti,* whilst in the present species it occupies a midway position. As regards the male caudal papillae comparison is possible only between *A. bihamata* and *A. madagascariensis.*

Family: Acuariidae Seurat, 1913.
Subfamily: Acuariinae Railliet, Henry and Sisoff, 1912.
Genus: Streptocara Railliet, Henry and Sisoff, 1912.
Streptocara sp. inq. (Figs. 25-26.)

This material consists of three females only which were collected from the proventriculus of *Spheniscus demersus* from S. Africa. These



Ancyracanthopsis madagascariensis n.sp.
21. Lateral view of anterior end of body. 22. Dorsal view of anterior end of body.
23. Lateral view of male tail. 24. Lateral view of female tail.

Streptocara sp. inq.
25. Dorso-lateral view of anterior end of body. 26. Lateral view of female tail.
Scale .05 mm. refers to Figs. 21 and 22.
Scale .1 mm. refers to Figs. 23 and 24.

filiform worms measure 12.12-15.15 mm. long by 0.232-.245 mm. wide, and are much attenuated from the level of the middle of the glandular oesophagus to the vestibular region where it is 0.030-.058 mm. wide.

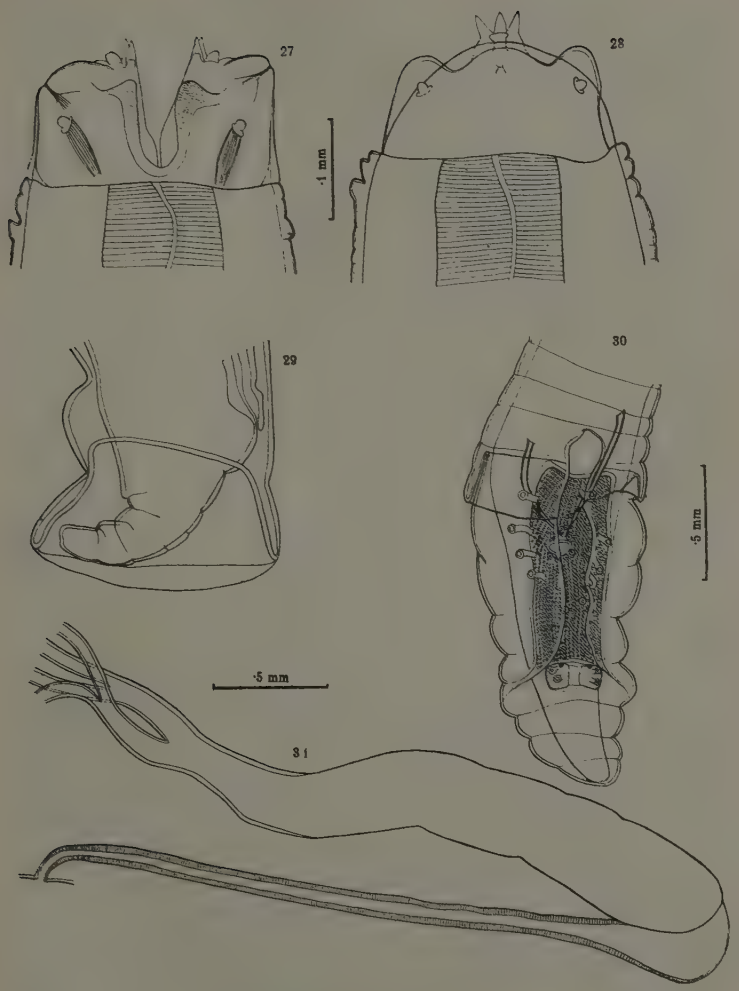
The rest of the body is more or less uniform in thickness. The body cuticle is finely striated transversely throughout. The mouth consists of two triangular lateral lips, each having a cuticular tooth at the mid-summit and two submedian papillae at both the dorsal and ventral corners but no lateral papillae were seen. The head is armed with a "collar" in the form of epaulettes whose free edge is fringed with posteriorly directed spines. The two "cervical papillae" are situated about 0.043 mm. from the anterior extremity, each having 5-7 posteriorly directed cusps at its free end. The vestibule is cylindrical with a transversely striated wall, 0.029-.0.031 mm. long. The oesophagus measures 3.13-3.51 mm. or about a quarter of the body length; the anterior muscular part is 0.516-.642 mm. long by 0.044-.046 mm. wide. The nerve ring and excretory pore are 0.133-.140 and 0.163-.195 mm. from the anterior end respectively. The bluntly rounded tail is 0.032-.042 mm. long, and the anus is subterminal. The vulva is situated at 8.07-9.16 mm. from the anterior end; it opens into a muscular vagina 0.61 mm. long which runs backwards as well as dorsalwards, turns upon itself and proceeds forward. The uteri are fully distended with embryonated eggs. They are thick shelled and measure 0.032-0.036 × .016-.017 mm.

The present material together with *S. crassicauda* (Creplin,1829) Skrjabin, 1916, and *S. crassicauda* var. *charadrii* Skrjabin, 1916, and *S. recta* (Linstow, 1897) Skrjabin, 1916, forms a group interrelated by the similarity in the denticulate "collar", in the "cervical papillae" and in the shape of the female tail. In the absence of the males it is difficult to make a specific diagnosis but it differs from *S. crassicauda* in the relatively posterior position of the vulva and apparently in the cylindrical form of the vestibule. It should be noted that the host of the present material is different from those of the other species.

Family: PHYSALOPTERIDAE Leiper, 1908.
Subfamily: PHYSALOPTERINAE Railliet, 1893.
Genus: PHYSALOPTERA Rudolphi, 1898.

Physaloptera singhi n.sp. (Figs. 27-31.)

This material consists of numerous specimens of both sexes, of which a good many are sexually mature. Though the label on the bottle is indecipherable, it is assumed that they were collected from the alimentary canal of a monkey in S. America, since they bear most resemblance to the simian species of this genus.



Physaloptera singhi n.sp.

27. Dorsal view of anterior end of body. 28. Lateral view of anterior end of body.
29. Lateral view of female tail. 30. Ventral view of male tail. 31. Female genitalia.
Scale .1 mm. refers to Figs. 27 and 28.
Scale .5 mm. refers to Figs. 29, 30 and 31.

Only sexually mature specimens were examined. The female is 29.3-35.5 mm. long by 1.2-1.7 mm. wide and the male 17.1-20.4 mm. long and 0.59-.64 mm. wide. The body cuticle is annulated, the annulations varying in size in different parts of the body but usually averaging 0.1 mm. and 0.068 mm. in width in the middle of the body of the female and male respectively. Within each annulation there are fine transverse striations. In the majority of the specimens of both sexes the cuticle is not reflected over the lips so that no cephalic collar is formed. In the female it is always reflected over the tail to form a cylindrical sheath. In the male this sheath may cover as much as the anterior half of the caudal region and extends farther back on the right side than on the left. At the anterior extremity there are six cephalic papillae, four prominent submedians and two inconspicuous laterals. The external median tooth is conical with an obtuse tip slightly bending outwards and smaller than the inner median one; the inner tooth gives rise to three large conspicuous cusps. The oesophagus varies from 5.57 to 5.76 mm. and from 3.52 to 4.13 mm. long in the female and male respectively; the anterior muscular part measures 0.57-.76 mm. long by 0.162-.171 mm. wide in the female and 0.514-.57 mm. and 0.126 mm. respectively in the male. The nerve ring encircles the posterior fourth of the muscular oesophagus. The excretory pore is situated at 0.81-.95 mm. and 0.67-.76 mm. from the anterior end of the female and male respectively. The cervical papillae are situated a little behind the anterior end of the glandular oesophagus.

The female: The finger-like tail bends dorsally and is enclosed by the cylindrical reduplicated sheath. The vulva is inconspicuous and is situated in the middle third of the body about 11.7-15.2 mm. from the anterior end. It opens into a thick-walled vagina about 2.2-2.6 mm. long, which runs forward and leads into a swollen egg-chamber. (Fig. 31.) The latter is 2.0-3.5 mm. long by 0.23-.35 mm. wide and opens into a short common trunk which is 0.38-.43 mm. long. Four uteri arise dichotomously from the common trunk, each dichotomy being 0.30-.39 mm. long. The uteri contain fully embryonated eggs.

The male: The tail measures 1.14-1.33 mm. long. Its medio-ventral surface is ornamented with numerous oval tubercle-like bodies arranged in longitudinal rows in front of the anus to the level of the terminal end of the caudal alae. The alae are prominent, 1.14-1.24 mm. long, and are united in the mid-ventral line about 0.47 mm. in front of the anus where the presence of the caudal sheath causes them to be folded

inwards. There are four pairs of lateral circumanal pedunculate papillae of which the posterior three are grouped together around the anal region while the first pair is a little distance in front of the anus. On the medio-ventral surface of the subterminal region there are two pairs of short pedunculate papillae situated in a slightly raised rectangular area at the level of the terminal end of the caudal alae. Three sessile papillae are arranged in a triangular position immediately in front of the anus and four sessile ones are arranged in a row closely adjacent to the posterior rim of the anus. A pair of ventral sessile papillae is situated in the anterior third between the anus and the tail tip. There is a pair of very small sessile papillae mid-way between the anus and the tail tip or immediately in front of the raised rectangular area. The right and left spicules vary from 0.35-0.52 mm. and from 0.51 to 0.69 mm. long respectively. In some specimens they are subequal, the difference in length being only 0.05 mm. The right spicule is slightly curved with a blunt end while the left one ends in a point.

As far as can be ascertained, five species of this genus provided with a prepuce-like sheath over the tail have been reported from carnivora and monkeys, viz. *P. praeputialis* Linstow, 1889, *P. malayense* Ortlepp, 1922, *P. canis* Mönnig, 1929, *P. tumefaciens* Henry and Blanc, 1912 and *P. lagothricis* (*Chlamydonema lagothricis* Kreis, 1945). The present species has morphological affinities with *P. tumefaciens* and *P. lagothricis*, but it differs from them as follows: In the first place, the four uteri arise dichotomously from the common trunk. Secondly, its vulva is at the middle third of the body instead of at the anterior fourth. Thirdly, on the ventral surface of the subterminal region of the male tail of the new species there are two pairs of short pedunculate papillae which are absent in the others. Furthermore, according to the description by Kreis, *P. lagothricis* appears to have no external median teeth on the lips.

In passing it must be mentioned that the genus *Chlamydonema* was created by Hegt (1910) to separate the species with a prepuce-like sheath over the posterior extremity, from the other members of the genus *Physaloptera*. Subsequently it was adopted by some helminthologists. But this morphological feature appears to the writer to be of specific rather than of generic rank and therefore these species are retained in the genus *Physaloptera*.

I have pleasure in naming the new species after my fellow-worker in helminthology, Mr. S. N. Singh.

Physaloptera sp. inq. (Figs. 32-37.)

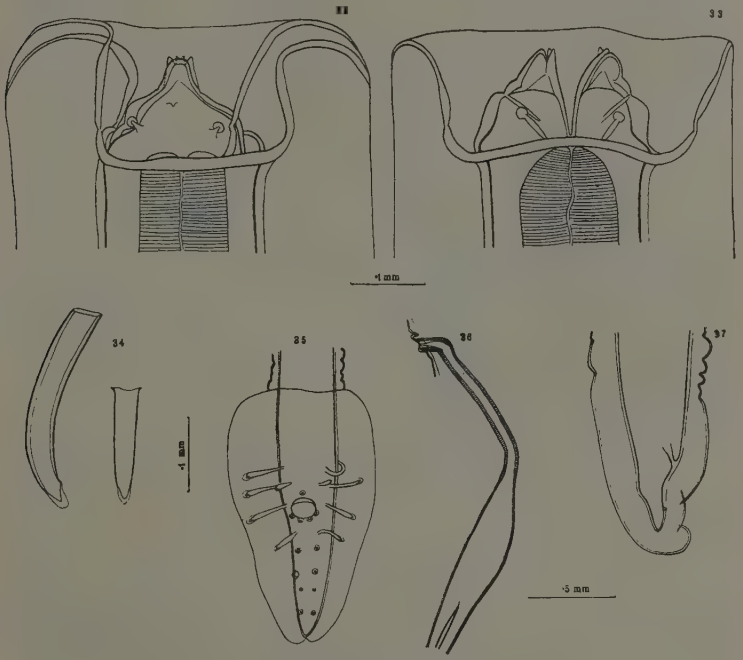
This materal consists of a good many specimens of both sexes collected from the stomach of the silver mongoose (*Herpestes cafra*) in S. Africa. The body cuticle of these specimens shows some degree of disintegration probably owing to the change brought about after the death of the host. In this connection it is worth while mentioning that another bottle of *Physaloptera* collected from a mongoose could not be identified specifically because the specimens had become blackened too much for detailed examination.

They measure 27.67-33.02 and 18.06-20.10 mm. long by 0.619-.704 and 0.34-.43 mm. wide in the female and male respectively. The body is attenuated towards both the anterior and posterior extremities. The body cuticle is very finely striated transversely. Anteriorly it is reflected over the head to form a collar. The two lateral lips are triangular in shape, each bearing an external and an internal median tooth. The external one is strikingly massive, about 0.048 mm. long and 0.042 mm. in maximum width, with a round tip; the inner tooth is almost as big as the outer and terminates in three equal cusps, the middle one of which usually gives rise to two small projections at the tip. Six papillae (four prominent submedians and two inconspicuous laterals) are present on the lips. The total length of the oesophagus is 5.93-6.79 and 4.95-5.04 mm. in the female and male respectively; the anterior muscular part is much shorter than the posterior glandular part which is 5.42-6.28 mm. long by 0.28-0.29 mm. wide in the female, the corresponding measurements being 4.44-4.52 mm. and 0.22-.23 mm. respectively in the male. The excretory pore and cervical papillae are situated nearly at the same level shortly behind the anterior end of the glandular oesophagus. The nerve ring is at 0.40-0.43 mm. and 0.38-0.42 mm. from the anterior end of the body in the female and male respectively.

The female: The tail is short, conical, 0.35-0.44 mm. long, with a pair of papillae at 0.111-0.135 mm. from the tip. The vulva may be either anterior or posterior to the end of the oesophagus; in ten females examined the vulva is post-oesophageal in seven, and varies from 0.22 to 1.36 mm. posterior to the end of the oesophagus, while it is 0.628-1.0 mm. anterior to the end of the oesophagus in the remainder. The muscular vagina is 0.99-1.45 mm. long, and passes straight backwards to communicate with the wide egg-chamber which is 0.57-.69 mm. long by 0.19-0.23 mm. broad and usually contains many eggs. Two uteri arise directly from the base of the egg-chamber, a common trunk

being absent. The thick shelled eggs in the uteri are about 0.051 × 0.037 mm. and contain a fully developed embryo.

The male: The well developed caudal alae are 1.33-1.62 mm. long. The ventral as well as the lateral surface of the caudal region is ornamented with longitudinally arranged rows of cuticular beads which



Physaloptera sp. inq.
32. Lateral view of anterior end of body. 33. Dorsal view of anterior end of body. 34. Two spicules. 35. Ventral view of male tail. 36. Female genitalia. 37. Lateral view of female tail.
Scale .1 mm. refers to Figs. 32 and 33.
Scale .5 mm. refers to Figs. 35, 36 and 37.

disappear in the most subterminal region. Of the four circumanal lateral pedunculate papillae, two are preanal, one adanal, and one postanal, the last pair being the shortest of all. Immediately in front of the anus there are on the ventral surface three papillae arranged in a triangle, the antero-laterals of which are pedunculate; the posteromedian one is sessile. The posterior margin of the anus is studded with

four sessile papillae arranged in a row. There are also on the ventral surface of the subterminal region four pairs of sessile papillae, the third pair of which is the smallest. The left and right spicules are 0.172-0.299 mm. and 0.11-0.229 mm. long respectively; as a rule the left spicule is longer than the right but in some specimens it is a little shorter. They are robust and somewhat thimble-shaped, with a cap at the distal ends.

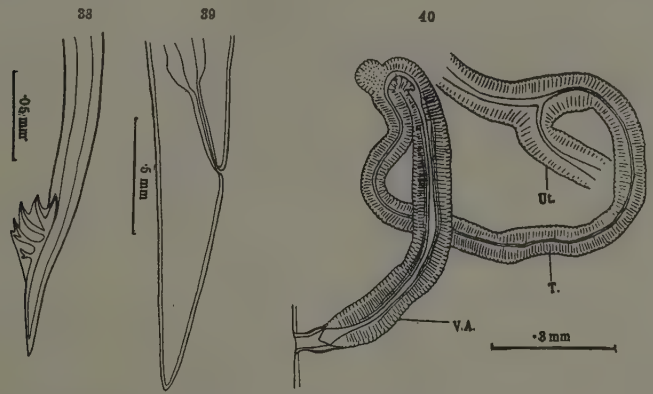
The present species together with *P. dispar* Linstow, 1904 (syn. *P. clausa* of Seurat, 1917), *P. tacapensis* Seurat, 1917 and *P. immerpani* Ortlepp, 1937, constitute a group inter-related by their similarity in the caudal ornamentation as well as in the arrangement of the caudal papillae in the male, in the mode of the origin of the uteri and in the shape of the two short spicules. According to Baylis (1928) who examined the type specimens of *P. dispar* and compared them with the description of *P. clausa* given by Seurat (1917) the two species are identical. Through the courtesy of Dr. H. A. Baylis the writer was kindly given the opportunity of examining the type specimens of *P. dispar* in the British Museum. The writer is convinced that the present species differs from *P. dispar* especially in the shape of the head, in the strikingly big teeth and in the shorter spicules.

As regards the relationship between *P. clausa* of Seurat and *P. tacapensis,* the writer agrees with Ortlepp (1923) that from Seurat's description the latter species appears to be so closely related to his *P. clausa* that their separation is hardly warrantable.

The relationship of the present species to *P. immerpani* cannot yet be established since it depends largely upon an accurate comparison of the lips and the teeth. Ortlepp's description of the lips and teeth of *P. immerpani* might equally well fit the present species but in the absence of a figure of these structures in *P. immerpani* it cannot be concluded with any certainty whether they are identical or not. In a personal communication, Dr. Ortlepp informs me that the type specimens of his species are unfortunately decomposed, so that a direct comparison is now impossible. Other differences between the two species which have been noted are as follows: (1) the spicules are longer in Ortlepp's species than in the writer's and the left one is slender in the former while it is more robust in the latter; (2) the vulva is anterior to the posterior end of the oesophagus in Ortlepp's species but varies in position in the present one.

Habronema mansioni Seurat, 1914.

This species was obtained from *Dryobatus major anglicus* in England and agrees in essentials with the description of *Habronema mansioni* but differs in the length of the left spicule which averages 1.05 mm. instead of 0.68 mm. Gendre (1922) (see in Cram, 1927) and Li (1934) also found that the left spicule was longer in their specimens. In this connection it is worth mentioning that in the present material the lateral body alae are unequal, one extending almost to the middle of the body and the other narrower and extending to about the anterior third of the body.



Hartertia zuluensis Mönnig, 1931

38. Distal end of left spicule. 39. Lateral view of female tail. 40. Female genitalia.

VA—Vagina, T—" Trompe ", UT—Uterus.

Physaloptera paradoxa Linstow, 1908.

Three females and three males were obtained from the stomach of *Varanus exanthematicus,* all of which were sexually mature. They conform in important features to the description of *Physaloptera paradoxa,* except that the four uteri arise dichotomously from the common trunk of the genitalia and there is an extra pair of small papillae next to the last pair on the ventral surface of the subterminal region of the male tail. An unnamed species was recorded from *Varanus indicus* by Mirza (1934) who was unable to decide whether it was *P. paradoxa* or *P. varani,* because the four uteri arise dichotomously as in *P. varani,* but the lips are provided with rows of denticles in a manner very similar

to *P. paradoxa*. The present material is identical with that of Mirza except for the apparent absence of lateral cephalic papillae and it is probable that both represent a hostal variety of *P. paradoxa*.

Hartertia zuluensis Mönnig, 1931. (Figs. 38-40.)

Mönnig (1931) described this species only on the basis of a single male specimen from a bustard (scientific name not given) in Zululand, Africa. Since then no female of this species has been described. Fortunately, a good many specimens collected from the water dikkop (*Burlinus vermiculatus*) and black korhaan (*Afrotis afra afra*) in S. Africa, were available for the present study. On examination they proved to agree in essentials with the description of *H. zuluensis* by Mönnig. Nothing more is added here to Mönnig's description of the male except a brief comment on the spicules. In the present material the left spicule measures 2.15-2.4 mm. long with four barbs near the distal end and the right one is 0.55-.59 mm. long with a membraneous cap over the distal round end. Mönnig stated that the left spicule is 2.89 mm. long with two barbs near the end and the right one is 0.868 mm. long.

The female measures 29-32 mm. long by 1.03-1.07 mm. wide. The body tends to curve dorsally, becomes attenuated towards both extremities and particularly towards the posterior end; its cuticle is prominently striated transversely. Cervical alae are not present. The anterior extremity bears two large lateral lips which appear to be separated from the body in the lateral view. They are each trilobed with a very small spike-like tooth on the inner surface of each lobe. Each lip has an inconspicuous lateral papilla and two submedian papillae. A little behind the laterals and almost at the level of the submedians there are two cervical papillae. The total length of the oesophagus is 4.24-4.35 mm. and it consists of two parts, the anterior muscular one being 0.67-0.81 mm. long. The nerve ring is at 0.47-0.57 mm. from the anterior end. The vulva is inconspicuous and situated at a distance of 12.14-13.56 mm. from the anterior extremity. It opens into a muscular vagina ("vestibule" according to Seurat) which is about 0.84 mm. long, runs forward and turns into the undivided part of the uterus (designated "trompe" by Seurat). The latter is about 1.43 mm. long and is divided into two uteri which run in opposite directions. The vagina has a cul-de-sac at its union with the "trompe". The thick shelled ova in the uteri are 0.042 × 0.035 mm. and contain a fully

developed embryo when oviposited. The tail is long, slender, and the anus is 0.96-1.05 mm. from the tip.

Summary.

Six new species of Spirurids are described from terrestrial vertebrates from various countries. They are *Camallanus mazabukae* n.sp. and *Procamallanus brevis* n.sp. from bull-frogs in S. Africa, *C. multilineatus* n.sp. from *Rana catesbiana* in N. America, *Tanqua gigantica* n.sp. from *Python reticulatus* and *Naja hannah* in E. Indies and S.E. Asia, respectively, *Ancyracanthopsis madagascariensis* n.sp. from *Dryolimnas cuvieri* in Madagascar, and *Physaloptera singhi* n.sp. from the monkey (?) in S. America. Three species inquirendae, *Camallanus* sp., *Streptocara* sp. and *Physaloptera* sp. are also described. Some new morphological features in *Habronema mansioni* and *Physaloptera paradoxa* are discussed and the female of *Hartertia zuluensis* is described for the first time.

Acknowledgments.

The writer wishes to acknowledge with thanks the assistance provided him by members of the staff of the Department of Parasitology and to express his gratitude to Professor J. J. C. Buckley for constant supervision and advice. He is also indebted to Dr. P. Leroux for helpful criticism and for presenting some of the African material described herein. Thanks are due to Professor H. E. Shortt, Director of the Department of Parasitology, for facilities for working in the Department and to Mr. F. R. N. Pester for his valuable assistance. The kind help and courtesy of Dr. H. A. Baylis and Dr. R. J. Ortlepp are also gratefully acknowledged.

References

Baylis, H. A., 1928.—" On a collection of nematodes from Nigerian mammals (chiefly Rodents)." *Parasitology*, XX (3), 280–304. (*W.L.* 16035.)

Cram, E. B., 1927.—" Bird parasites of the nematode suborders Strongylata, Ascaridata and Spirurata." *Bull. U.S. nat. Mus.*, No. 140. (*W.L.* 5659.)

Diesing, K. M., 1861.—" Revision der nematoden." *S. B. Akad. Wiss. Wien.*, XLII, 595–736. (*W.L.* 20170.)

Drasche, R., 1884.—" Revision der in der Nematoden-Sammlung des k. k. zool. Hofcabinetes befindlichen Original-Exemplare Diesing's und Molin's." *Verh. zool.-bot. Ges. Wien.*, XXXIII, 107–118. (*W.L.* 22327.)

Hegt, J. N., 1910.—" *Chlamydonema felineum* nov. gen., nov. spec., eine neue parasitisch lebende Nematode." *Tijdschr. ned. dierk. Ver.*, ser. 2, pt. 12, Leiden. (*W.L.* 21275.)

Kreis, H. A., 1945.—" Beitrage zur Kenntnis parasitischer Nematoden. XII. Parasitische nematoden aus den Tropen." *Rev. suisse Zool.*, LII (4), 551–596. (*W.L.* 19288.)

Li, H. C., 1934.—" Report on a collection of nematodes mainly from N. China. Part II.—Spiruroidea." *J. R. micr. Soc.*, LIII (2), 174–195. (*W.L.* 11360.)

Mirza, M. B., 1934.—" *Sciuris palmarum* als ein interessanter Wirt von *Physaloptera* sp." *Z. Parasitenk.*, VI (5), 638–640. (*W.L.* 23536a.)

Molin, R., 1860.—" Trenta specie di nematoidi." *S. B. Akad. Wiss. Wien.*, XL, 331–358. (*W.L.* 20170.)

Mönnig, H. O., 1931.—" Two new species of the nematode genus *Hartertia*." *17th. Rep. Dir. vet. Serv. and Anim. Indust. Pretoria*, 273–276.

Müeller, A., 1897.—" Helminthologische Mittheilungen." *Arch. Naturgesch.*, LXIII, 1–26. (*W.L.* 1782.)

Ortlepp, R. J., 1923.—" The nematode genus *Physaloptera* Rud." *Proc. zool. Soc. Lond.*, 999–1107. (*W.L.* 16737.)

Seurat, L. G., 1917.—" Physalopteres des mammifères du Nordafricain." *C. R. Soc. Biol. Paris.*, LXXX, 210–218. (*W.L.* 6630.)

Southwell, T. & Kirshner, A., 1937.—" On some parasitic worms found in *Xenopus laevis*, the S. African clawed toad." *Ann. trop. Med. Parasit.*, XXXI (2), 245–266. (*W.L.* 1063.)

Walton, A. C., 1935.—" The nematoda as parasites of Amphibia." *J. Parasit.*, XXI (1), 27–50. (*W.L.* 11428.)

Journal of Helminthology, Vol. XXII, Nos. 3/4, 1948, pp. 165—178.

*On the Transverse Fission of *Cysticercus pisiformis* in experimentally infested Rabbits, and the Phylogenetic Significance of Asexual Phenomena in Cysticerci.

By Hilary Crusz, Ph.D., B.Sc.

Assistant Lecturer in Zoology, University of Ceylon.

(From the Department of Parasitology, London School of Hygiene and Tropical Medicine.)

Moniez (1880) in his classical monograph on the cysticerci gave an account, illustrated by one figure, of a stage in transverse division which he observed in 22-days-old larvae of *Taenia pisiformis*. He did not, however, trace in detail the anatomical or histological changes involved in this process. His work has not hitherto been confirmed and has not been generally accepted by parasitologists. Solomon (1934), in an attempt to reinvestigate the problem, came to the conclusion that "the observations of Moniez in this matter are either erroneous or descriptive of an isolated or abnormal phenomenon".

The present writer decided to investigate this point in the material which he obtained from experimental infestations of rabbits with gravid proglottides of *Taenia pisiformis*. As many of the cysticerci in the liver and body-cavity were found to be in various stages of transverse fission, and as some cysticerci that appeared to be entire showed evidence of having already undergone fission, a detailed study of this process was made.

Material and Methods.

A dog infested with *Taenia pisiformis* was very kindly placed at the author's disposal by Dr. Mary Franklin of Rothamsted Experimental Station. Two laboratory-bred rabbits, A and B (two-months-old bucks), were infected orally with one gravid proglottis each, obtained from the dog's faeces. Two days later the dog was treated with the proprietary arsenical anthelmintic "Nemural" by Mr. J. W. G. Leiper, M.R.C.V.S., and it passed one complete tapeworm, a study of which confirmed the specific diagnosis. Rabbits A and B were each given another gravid proglottis, and a two-months-old laboratory-bred doe (rabbit C) was given four gravid proglottides, in a single feeding. It was originally intended to sacrifice these animals on the 18th, 24th and 31st days after the administration of proglottides, but rabbits A and B died 16 and 23 days respectively, after the second feeding, and

*Part of a thesis approved by the University of London for the award of the Ph.D. Degree.

rabbit C was sacrificed on the 31st day as originally intended. All the rabbits showed heavy infestation of liver and body-cavity with bladder-worms of this species. The lungs too were found to be infested with a few of them. General observation showed that the bladder-worms in the body-cavity of the three rabbits were at three distinctly different stages of development, but closer study revealed a fair amount of overlapping in regard to the earlier stages, so that even with material from rabbit C alone, one was able to make a more or less complete study of the development of bladder and scolex from the early stages passed in the liver right up to the time of differentiation of rostellum, suckers and hooks.

For detailed histological study, the livers were cut up into pieces of suitable size, washed in physiological salt solution and fixed in various fluids. The free cysticerci of the body-cavity and those enclosed in the mesenterial folds were also collected: some of them were fixed after being washed in physiological salt solution, while others were plunged directly into the fixing fluid. The fixatives used were Carnoy (6 : 3 : 1), Helly, Petrunkewitsch, Bouin, formal-saline and 10% formalin. Material was embedded in paraffin, and serial sections were cut at a thickness of 4μ, 6μ and 8μ and stained in Heidenhain's iron haematoxylin with or without eosin, chlorazol black E and Mallory's triple stain. Carnoy and Helly proved to be the best fixatives. Iron haematoxylin was, as usual, unrivalled for the wealth of cellular detail it afforded. Mallory's triple stain gave fine pictures of tissues differentially stained: the cuticle stained a distinct blue in contrast to the muscle-layers and subcuticular cells which stained bright red. Chlorazol black E proved to be a very good general stain for cestode tissue. It stained almost as well as Heidenhain's haematoxylin, especially after Carnoy fixation, and was easy to use.

Although the greater part of the material was fixed for histological study, much of it was examined in the living condition before fixation, and the general behaviour and movement of the bladder-worms within the liver (in macerated pieces), on its surface and in the body-cavity, were carefully observed. Whole mounts of some of the fixed material, stained in borax carmine or eosin, were made for anatomical study.

Description.

Cysticerci teased out of liver tissue and examined under a binocular microscope, but more often studied from serial sections of liver, show considerable variation in length and state of development. They are all over a millimetre long and may attain a length of 6-8 mm. before leaving

the liver. It is only in their general shape and histological features that they show some degree of constancy. They are narrow, elongate structures, which lie stretched out, bent or contracted, in the substance of the liver, no doubt an indication of their wriggling movements which are easily observed in living, macerated tissue. One end of the bladder-worm is distinctly broader than the other (Fig. 1a) and is recognized as the anterior end which develops the scolex-anlage. Stained sections through any of these larvae reveal the typical structure long well-known (Young, 1908). Investing the entire bladder-worm is a thin, distinct

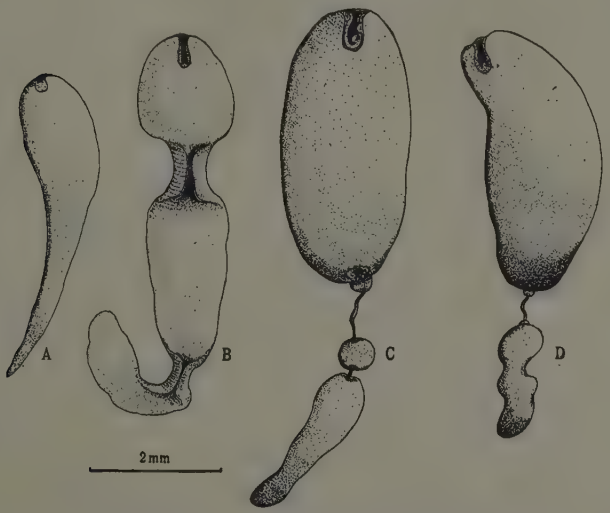


Fig. 1. *Cysticercus pisiformis* from body-cavity of experimentally infested rabbit A. *A*. Young cysticercus before fission. *B-D*. Stages in transverse fission.

cuticle, which is straight and homogeneous and is covered for the most part by fine, closely set hair-like processes. It may show characteristic undulations in regions where there has been contraction of the bladder-wall. Below the cuticle is a layer of circular muscle fibres followed by a layer of longitudinal muscle fibres. Then follow the subcuticular parenchymatous cells, which in the younger larvae occupy the greater portion of the bladder-cavity. These cells have large vesicular nuclei and their cytoplasm is drawn out into long thin processes which anastomose freely with those of neighbouring cells, thus forming the reticular

parenchymatous syncytium, typical of larval cestodes in early stages of development. Nearer the cuticle these parenchymatous processes are seen to traverse the region of the muscle layers and to reach the cuticle. From the earliest stages of development there is in the centre of the cysticercus a small bladder-cavity which enlarges as the larva grows larger, until the subcuticular parenchyma becomes reduced to a narrow zone of anastomosing cells beneath the muscle layers. (Plate 1a). Larvae at this stage occur in plenty, especially in Rabbit A, not only within the liver but on its surface as well, lying within the serosa, and in the body-cavity. (Fig. 1a).

The process of transverse fission apparently begins in the liver and may be completed within the liver or in the body-cavity. The onset of fission is marked by a characteristic annular constriction which may occur anywhere behind the region involved in the formation and protection of the scolex-anlage. There may be one or two constrictions, (Fig. 1b) resulting in the fission of the original cysticercus into two or three parts respectively, so that the larvae do not necessarily divide into two equal parts (Moniez, 1880). The extent of the zone of constriction may also vary from about a tenth of a millimetre to anything up to a millimetre or more. These constrictions are as interesting in living larvae as they are in serial sections, for they are opaque and whitish and show very limited powers of movement. The portions of the bladder-worm which they eventually cut off as separate units show the usual movements of normal bladder-worms. This fact shows that the constrictions are not mere contractions of the larva in the ordinary course of movement, but are actually a stage in transverse fission. The histology of these zones of transverse division proves this beyond doubt (Fig. 2a, Plate 1b). The cuticle, circular muscles and longitudinal muscles in these regions appear very much folded and thickened, due to actual hypertrophy accompanied by contraction of the body-wall: a process analogous to that which occurs in scolex-formation. The most interesting features, however, are the increase in number and size of the subcuticular cells and the general increase and characteristic orientation of the parenchyma fibres around the narrow bladder-cavity at the point of constriction. All this points to the gradual solidification of the constricted regions, which is easily recognized in sections. The subcuticula in these regions becomes specially transformed. It becomes many cells deep and the cells assume the elongate form and granular structure characteristic of older cestode tissue. Some of them have the

appearance of "gland-cells" and there are also to be seen large, granular, irregularly-shaped masses, devoid of deeply staining nuclei but still invested by a thin limiting membrane (Fig. 2a, Plate 1b). These lie nearer the bladder-cavity and give the impression that they are degenerating cells that are being released from the general mass of the subcuticula. The parenchyma fibres in this region are more numerous and thicker and lie in an antero-posterior direction.

As fission proceeds the constriction deepens and three important and easily recognizable processes take place, namely, the swelling and degeneration of the cuticle, the breakdown of the subcuticula, and the increase in number and gradual consolidation of all the fibrous elements in this region (Fig. 2b). The breakdown of the cuticula is interesting (Fig. 2b and c, Plate 1c). It swells considerably and assumes the vacuolated form so characteristic of cuticle in regions where fusion of its substance occurs, as in the developing rostellum (Gläser 1909, Crusz 1947). There is no doubt that the cuticle here is in a sort of "liquefied" condition. The subcuticular parenchyma has broken down considerably by now, and many of its nuclei are seen lying in granular cytoplasmic masses where cell boundaries are no longer distinctly visible. The muscle fibres and parenchyma fibres, in fact, are seen to have increased further and they begin to pack the zone of constriction and radiate out anteriorly and posteriorly into the respective halves of the original bladder-worm. These fibres are definitely in connection with the subcuticular parenchymatous fibres of the anterior and posterior halves.

The last stage, before the final separation of the parts of the original cysticercus, is represented by the segments still joined together by a slender musculo-fibrous cord, which is all that is left of the original zone of fission. (Fig. 1c and d). The histology of a bladder-worm at this stage (Fig. 2c, Plate 1c) amply confirms what has hitherto been described from cysticerci in earlier stages of fission. The cord is definitely fibrous and appears twisted in some cases. It is surrounded by a mass of broken-down subcuticular tissue, bits of cuticle and some liver debris. In fact, a few host cells are found to be scattered here and there over the entire surface of the bladder-worms, even on those parasitizing the body-cavity, which indicates that fission actually begins and is most often completed in the liver, as sections of liver also clearly reveal. The fibres of the cord are very tightly packed together and comprise numerous parenchymatous fibres, which form its bulk, and longitudinal muscle fibres. The latter undoubtedly enter into the make-up of the cord, for the longitudinal muscle fibres of the segments are

seen to be continuous with the outermost fibres of the cord. This would suggest that the cord itself retains its power of movement to the last, but observations of living specimens revealed nothing on this point owing to the small size of the cord and the overwhelming movements of the segments themselves.

Sooner or later the cord snaps, resulting in the release of the segments joined by it. This occurs within the liver or in the body-cavity. Such freed bladder-segments are easily identified by the presence of portions of the original cord at the ends involved in fission, or in case the cord has disappeared, by the specific histological change that has come over these ends. Such bladder-segments are also invariably short: some carry the remains of the cord, while others have only somewhat pointed thickenings which are the sole indication of the fact that fission has occurred. The histological features of these ends are the same as those described for the previous stage. In one instance a bladder-worm was found to have divided into three parts in the liver. Two segments, joined by a cord, were still within the liver, while the third had already left it. The end of that segment which was originally in connection with the freed segment shows in section not only the characteristic disposition of the numerous fibrous elements, but also a markedly swollen cuticle fusing into a "frothy" mass, apparently sealing up the broken end. The segments resulting from fission thus become entirely independent units. The anterior segment of the original bladder-worm continues to develop its scolex (Fig. 1), the rudiment of which normally appears long before the onset of fission. In no case was a scolex-anlage found in the posterior segment or segments.

Cysticerci in these stages of transverse fission were found in all three rabbits. The process was very easily recognized in Rabbit A, both in and out of the liver. In Rabbits B and C, the liver showed many cases of fission, especially among cysticerci that were leaving it and wriggling on its surface where small constricted bladder-worms were seen lying below the serosa. Cysticerci in earlier stages of fission were generally the most numerous. As regards cysticerci which had divided and whose segments were still connected by fibrous cords, more than twenty such were collected from the body-cavity alone of all three rabbits. The majority of the bladder-worms in the body-cavity showed no signs of having undergone any fission at all.

Before discussion of the details described above, the histological changes of the liver, brought about by parasitization with *C. pisiformis*, ought to be considered. This has been studied extensively by previous

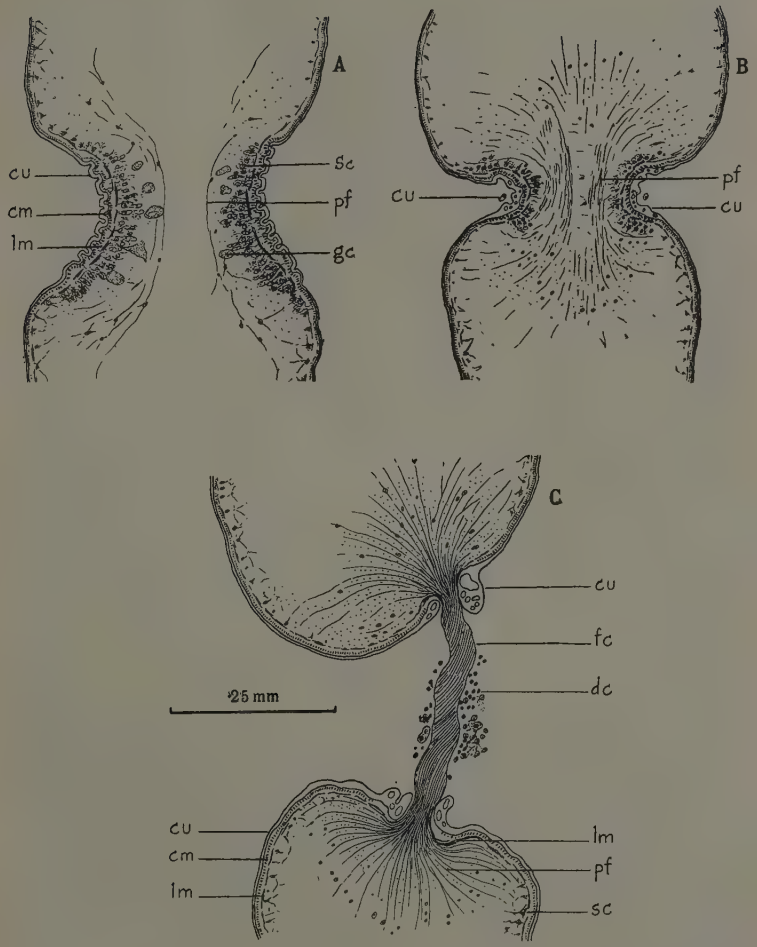


Fig. 2. Longitudinal sections through constricted regions of *C. pisiformis* at three different stages in transverse fission. (Semi-diagrammatic). *cu*, cuticle ; *cm*, circular muscle fibres ; *lm*, longitudinal muscle fibres ; *sc*, subcuticular parenchymatous cells ; *pf*, parenchyma fibres ; *gc*, granular cells ; *fc*, fibrous cord ; *dc*, degenerating cells.

workers (Moniez 1880, Solomon 1934, Leonard & Leonard 1940, Jirina 1943) and is fully confirmed by the present work.

The cavities in the liver, formed by the active larvae, contain large numbers of leucocytes (lymphocytes, eosinophils and some polymorphs) and necrosed liver cells, some of which appear to be in groups or clusters. Around this loose mass are more liver cells which are more or less typically aggregated. Then follows a narrow zone of fibrosis, which in turn is surrounded by healthy liver cells. The breakdown of hepatic tissue thus appears to occur in an extraordinary degree when compared with the reaction of rat's liver to infestation with *C. fasciolaris*. Nothing comparable to the well-developed fibrous cysts formed round the cysticerci of the rat, has been observed in rabbits' liver infested with *C. pisiformis*. The characteristic breakdown of liver tissue in the latter case, and the lack of well-defined and well-developed adventitious cysts, are probably correlated with the striking degree of development of the musculature and with the powers of movement this bladder-worm enjoys from its earliest stages of development, as compared with *C. fasciolaris*, where there are no signs of the characteristic musculature for a relatively long time during early development. The living intrahepatic larva of *C. pisiformis* is therefore relatively unhampered by any encroachment upon it of fibrotic elements of a constricting or otherwise limiting nature, and transverse fission appears to be, as Moniez made out, a spontaneous process.

Discussion.

Literature does not record many instances of the occurrence of transverse fission in *C. pisiformis* or other cysticerci. Moniez (1880) was the first to describe the last stage of this process as it occurred in the cysticerci of three of the rabbits he infected experimentally. He rightly recognized the stage when the two segments are joined by a slender fibrous cord as the last stage of a process of transverse division of a single cysticercus. He also found that only a small number of bladder-worms showed no signs of having divided at all, and regarded the process as one of normal occurrence in this species at least. He did not find any instance where the posterior half of a cysticercus in division develops a scolex; but in one case he doubtfully regarded the anterior thickening of the posterior segment as a scolex-anlage, which, however, is highly improbable in view of the profound histological modification that end undergoes during fission. It is true that Moniez

did not give a precise histological account of the stage he described, but the facts he did bring to light have been amply confirmed by the present study.

In a recent histo-pathological study of rabbits naturally infested with *C. pisiformis,* Jirina (1943) states: "Bei zwei Kaninchen fand ich einige Cysticerken, die facettenartig miteinander verbunden waren; in jeder Facette befand sich ein Skolex. Bei dem einen Kaninchen war ein solches Gebilde, beim zweiten waren sieben solcher facettenartiger Gebilde vorhanden". He probably refers here to cases of transverse fission, in which case it is of special interest to know that each segment of a dividing cysticercus bore a scolex-anlage. More details, especially of a histological nature, would have been desirable, since it is difficult to say for certain what is the nature of the process he has described. It is also possible that invaginated or non-invaginated fibrous thickenings due to a previous fission, may have been mistaken for scolex-anlagen. Be that as it may, there is Bott's interesting account (1897) of asexual reproduction in *C. longicollis* which throws light on this question. He describes and figures a stage in transverse division which, histologically, is almost identical with one of the stages here described, with the exception that Bott found clear-cut examples of the development of scolex-anlagen in the posterior segments as well.

It seems clear therefore that transverse fission is a well-established feature of at least two larval taeniids, *C. pisiformis* and *C. longicollis.* It may well be of much wider occurrence. In *C. pisiformis,* at any rate, it seems to be neither an abnormal nor an isolated phenomenon. There is no reason to doubt that the posterior segments cut off by fission can sometimes develop a scolex, especially if the anlage has been laid down very early in development, even before fission begins. But there appears to be no correlation between transverse fission and bicephaly. Generally, the posterior segments degenerate without producing scolices. That transverse fission is a spontaneous process also seems clear from the facts. Parasitized liver shows no histological modification of such a character as even to suggest that the cause of the transverse division of bladder-worms is mechanical. At all stages of fission the cysticerci move freely in the liver and emerge from it to enter the body-cavity. There may be some physiological reason, probably chemical, for the occurrence of fission in some bladder-worms and not in the majority. If this is so one wonders why the majority of them do not divide, since the physiological, especially the chemical, environment in the liver seems to be so uniform. From this it appears that, whatever the

immediate physiological cause, the reaction is to a great extent intrinsic and some bladder-worms react by way of transverse fission, which is therefore, biologically at least, a spontaneous process. When one considers such cases as the fission of *C. longicollis* in the body-cavity and sub-cutaneous tumours (Bott, 1897), and the constriction of daughter-bladders *inside* the fluid-filled parent-bladder of a proliferating *C. tenuicollis* (Crusz, 1948a), the spontaneity of this process is something readily understandable, and transverse fission becomes just another feature of the behaviour of cysticerci, though it may be rare and not necessarily a mode of actual vegetative increase. This leads to the wider question of the significance of this and other modes of asexual increase in cysticerci.

The Phylogenetic Significance of Asexual Phenomena in Cysticerci.

The ability to develop many scolices from the bladder-wall, to exhibit endogenous and exogenous budding, to undergo fission and to develop fertile internal daughter cysts by vesiculation of scolices (Coutelen, 1946), seems to be characteristic of the coenurus-like form. The echinococcus, owing to its enormous development of cuticle and extraordinary powers of vegetative reproduction, represents a specialized type of coenurus. The coenurus has also the widest geographical distribution and host distribution, suggesting that it is really primitive. The echinococcus has even a wider distribution, but the differences do not appear to be significant. The cysticercus, on the other hand, has hitherto been regarded as the most primitive of the three types merely because it consists of a single bladder with a single scolex. But there must be some significance in the fact that cysticerci can, though only very rarely or occasionally, show polycephaly (Lüpke 1892, Southwell & Kirshner 1937, Dollfus 1938, Lent 1942, Clapham 1942*, Kuntz 1943), internal budding (Goeze 1782, Leuckart 1886, Crusz 1948a),

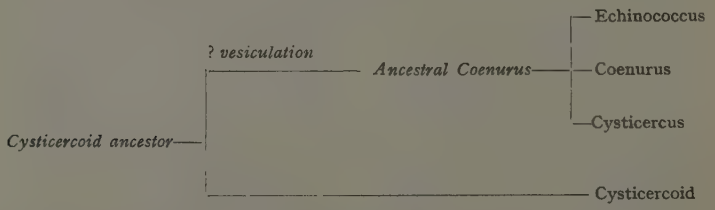
* In the writer's opinion, based on a study of the original material, *Multiceps macracantha* Clapham 1942 appears to be a polycephalic *Cysticercus fasciolaris*. Its hooks are typically those of the latter species. The differences seen in the guard and handle are in themselves insufficient to warrant the creation of a new species, as these parts are intraspecifically so variable in form and size. Clapham has overlooked the previous records of polycephaly in *C. fasciolaris* (Lüpke 1892, Southwell & Kirshner 1937, Dollfus 1938) from naturally infested rats and mice.

It is also worth noting here that in some recent experimental infestations of rats, with gravid proglottides of *Taenia taeniaeformis*, the writer discovered, in sections of the liver of one rat, a very young bicephalic *C. fasciolaris*. Each scolex-anlage of this specimen is an undifferentiated, bud-like mass of cells protruding from the bladder-wall.

external budding (Bremser 1824, Braun 1896, Bott 1897, Schwartz 1924, Penner 1938), and transverse fission (Moniez, 1880). All this, correlated with the fact that cysticerci show greater host specificity, points very strongly to the fact that the cysticercus, far from being the most primitive, is indeed the most specialized of the three types. This becomes all the more clear when we consider the nature of the larval bladder-wall as such. The ability of cysticerci to revert to the type of development shown by coenuri shows that the primitive bladder has the intrinsic power of producing scolices or budding or dividing at any point on its wall, and that one feature of specialization during evolution was the restriction of the area of scolex-formation to one point on the bladder-wall, and the loss or reduction of its powers of budding and fission, thus resulting in the cysticercus larva. This does not preclude the cysticercus displaying the primitive behaviour of the bladder-wall now and then, in response to circumstances not yet understood. From what has been said the cysticercus also appears to be more closely related to the coenurus than to the cysticercoid. It is not suggested that modern cysticerci have evolved directly from modern coenuri, but they have certainly evolved from a common coenurus-like ancestor. To put it more precisely, the primitive ancestral bladder-wall had greater potentialities of scolex-formation and vegetative increase than the bladder-wall of modern cysticerci, and, since their bladder-wall exhibits these potentialities to a greater degree, the modern coenuri are probably more primitive than the cysticerci. Biologically at least, it seems more probable that the vegetative increase shown so rarely by modern cysticerci is a reversion to primitive type than that it is a recent acquisition. Kuntz (1943), who favours Dew's "trauma theory" of the origin of daughter cysts in *Echinococcus,* regards all cases of polycephaly in cysticerci as a result of early injury. Here one is obviously looking for the physiological cause of the phenomenon, which does not necessarily explain the significance of the specific reaction, in this case, polycephaly, and in any case Dew's theory has been discredited by the brilliant researches of Dévé (1901-1946). One need only add that if the polycephaly of a coenurus could be regarded as a "genetically fixed" character, there is no reason why a forthright phylogenetic explanation should not be given for polycephaly and other vegetative phenomena in cysticerci.

If the view put forward be accepted, there remains the question of the relationship of the primitive coenurus to the cysticercoid ancestor. To visualize a polycephalic form as having directly evolved from a

cysticercoid seems, on the face of it, rather difficult, if not impossible. But, in the writer's view, at least two important clues should lead to a satisfactory answer to this question. The first is the ability of scolices to vesiculate, a fact so well established for *Echinococcus* by Dévé (1901-1946). The scolex of *Echinococcus*, which in so many respects resembles a cysticercoid, has the power of forming an entire, fertile daughter hydatid by vesiculation. According to Coutelen (1946) this occurs even in coenuri. Secondly there is Metschnikoff's discovery (1868) of an echinococcus-like cysticercoid (*Polycercus niloticus* Willey, 1907) from the body-cavity of an earthworm; and Haswell and Hill (1894) described an interesting *Polycercus* sp. which showed external proliferation from an original blastogen developed from the hooked embryo. Leuckart (1886) and Southwell and Prashad (1918) have reviewed these cases in detail. Leuckart put the matter briefly in a very significant paragraph: "The short description which we have given of this wonderful *Taenia*-form by no means exhausts the similarities which sometimes exist between the cysticercoids and the echinococci. The former are not only capable of internal proliferation but in some cases multiply also by external buds". This clearly shows that the first step in the evolution of the coenurus type from the cysticercoid ancestor need not necessarily have been *via* a bladder-worm with a single scolex, that is, a cysticercus. Indeed, it would seem to have been otherwise, but here more facts are needed before a satisfactory interpretation could be given. The supposed phylogeny of taeniids, from the point of view of their larval forms, may therefore be expressed as follows:—



Summary.

1. Transverse fission of *Cysticercus pisiformis* begins in the liver and is usually completed there. It is brought about by one or two annular constrictions which ultimately result in the complete fission of the original bladder-worm into two or three parts. The posterior segments thus formed are normally acephalic.

2. Histologically, fission involves an initial hypertrophy of the cuticle and subcuticula at the zone of constriction, followed by their degeneration and loss, and by the increase and consolidation into a slender cord of the muscle and parenchyma fibres. With the rupture of the cord and the sealing up of the broken ends, fission is complete.

3. The majority of the bladder-worms do not undergo fission, but the process is apparently spontaneous and quite normal for the species. Its cause is unknown, but it is thought to have a phylogenetic significance.

4. From a consideration of the known facts, the view is expressed that the occasional asexual multiplication of cysticerci is of such a nature as to indicate that the cysticercus has evolved from a coenurus type of ancestor rather than from a cysticercoid, and that modern coenuri, and even echinococci, are in that respect less highly evolved than cysticerci. It is suggested that from the available evidence it is not difficult to regard such a primitive ancestral coenurus as having evolved from a cysticercoid ancestor by a process analogous to the vesiculation of an echinococcus scolex.

Acknowledgments.

The writer is very grateful to Professor J. J. C. Buckley for the kind help and encouragement given throughout this work; to Professor R. T. Leiper, C.M.G., F.R.S., for constant help with the literature and many helpful suggestions; and to Dr. H. A. Baylis of the British Museum (N.H.) and Drs. T. Goodey, F.R.S. and B. G. Peters of Rothamsted Experimental Station, for their kind criticism and helpful suggestions in regard to the interpretation of the sectioned material.

References.

Bott, A., 1897.—" Über einen durch Knospung sich vermehrenden Cysticercus aus dem Maulwurf." *Z. wiss. Zool.*, LXIII, 115–140. (*W.L.*23635.)

Braun, M., 1896.—" Über einen proliferierenden Cysticercus aus dem Ziesel." *Zool. Anz.*, XIX, No. 514, 1–4. (*W.L.* 23833.)

1894–1900.—Cestodes: in Bronn's Klassen und Ordnungen des Thier-reichs. IV, Ib. *Leipzig.*

Bremser, J. G., 1824.—Icones Helminthum. *Wien.*

Brumpt, E., 1936.—Précis de Parasitologie. I. 5th Edit. *Paris.*

Clapham, P. A., 1942.—" On two new coenuri from Africa, and a note on the development of the hooks." *J. Helminth.*, XX, 25–31. (*W.L.*11224b.)

Coutelen, F., 1929.—" Essai de culture in vitro du cénure sérial, vésiculation des Scolex." *C.R. Soc. Biol.*, C. 619 (*W.L.* 6630.)

1946.—" Les modes de formation des vésicules filles externes et internes du Cénure sérial." *Ibid.*, 258–259.

Crusz, H., 1947.—" The early development of the rostellum of *Cysticercus fasciolaris* Rud., and the chemical nature of its hooks." *J. Parasit.*, XXXIII, 87–98. (*W.L.*11428).

1948a.—" Observations on a case of endogenous budding in *Cysticercus tenuicollis* Rudolphi." *J. Helminth.*, XXII, (*W.L.* 11224b.)

Dévé, F., 1946.—L'Echinococcose secondaire. *Paris.*

Dollfus, R., 1938.—" Sur un *Cysticercus fasciolaris* Rudolphi tératologique (polycéphale)." *Ann. Parasit. hum. comp.*, XVI, 133–141. (*W.L.* 899a.)

Fuhrmann, O., 1930–31.—Cestoidea : in Kükenthal u. Krumbach's Handbuch der Zoologie 2 : 1st Halfte (2).

Gläser, H., 1909.—" Zur Entwicklungsgeschichte des *Cysticercus longicollis* Rud." *Z. wiss. Zool.*, XCII, 540–561. (*W.L.* 23635.)

Goeze, J. A. E., 1782.—Versuch einer Naturgeschichte der Eingeweidewürmer thierischer Körper. *Blankenburg.*

Haswell, W. A., & Hill, J.P., 1894.—" On Polycercus : a proliferating cystic parasite of the earthworms." *Proc. Linn. Soc. N.S.W.*, VIII, 365–376. (*W.L.* 16790a.)

Jirina, K., 1943.—" Ueber die Cysticerkose der Kaninchen." *Berl. u. Münch. tierärztl. Wochenschrift u. Wiener tierärztl. Monatsschrift.* Jhrg. 1943, 261–262.

Korschelt & Heider, 1936.—Vergleichende Entwicklungsgeschichte der Tiere Erster Band. *Jena.*

Kuntz, R. E., 1943.—" Cysticercus of *Taenia taeniaeformis* with two strobilae." *J. Parasit.*, XXIX, 424–425. (*W.L.* 11428.)

Lent, H., 1942.—" Nota sôbre o encontro de cisticercos de *Taenia solium* L., 1758, com dois escôlices." *Revista Brasileira Biologia.*, II, 197–201.

Leonard, A. B. & Leonard, A. E., 1940.—" The cellular proliferation associated with cysticercus infestations in the cottontail rabbit." *Trans. Kans. Acad. Sci.*, XLIII, 429–432. (*W.L.* 21533).

Leuckart, R., 1886.—The parasites of man, and the diseases which proceed from them. Translated by W. Hoyle. *Edinburgh.*

Lüpke, F., 1892.—" Zweiköpfiger *Cysticercus fasciolaris*." *Repertorium der Thierhlkde.* Jhrg. 53. Hft. 9, 271–272. Stuttg. (*vide* M. Braun in Bronn's Klassen u. Ordnungen.)

Metschnikoff, E., 1868.—" Ueber eine Scolex-Colonie." *Arbeit d. l. russ. naturf. Vers. Petersb.* (*Zool.*), 263–266.

Moniez, R., 1880.—" Essai monographique sur les cysticerques." *Travaux de l'institut zool. de Lille.* III, Fasc. 1.

Penner, L. R., 1938.—" A hawk tapeworm which produces a proliferating cysticercus in mice." *J. Parasit.*, XXIV, Suppl., 25. (*W.L.* 11428.)

Schwartz, B., 1924.—"A new proliferating larval tapeworm from a porcupine." *Proc. U.S. Nat. Mus.*, LXVI, Art. 24, 1–4. (*W.L.* 16944.)

Solomon, S. G., 1934.—" Some points in the early development of *Cysticercus pisiformis* (Bloch, 1780)." *J. Helminth.*, XII, 197–204. (*W.L.* 11224b.)

Southwell, T. & Kirshner, A., 1937.—" Description of a polycephalic cestode larva from *Mastomys erythroleucus*, and its probable identity." *Ann. trop. Med. Parasit.*, XXXI, 37–42. (*W.L.* 1063.)

Southwell, T. & Prashad, B., 1918.—" Methods of asexual and parthenogenetic reproduction in cestodes." *J. Parasit.*, IV, 122–129. (*W.L.* 11428.)

Young, R. T., 1908.—" The histogenesis of *Cysticercus pisiformis*." *Zool. Jber.* Anatom., XXVI, 183–254. (*W.L.* 23835.)

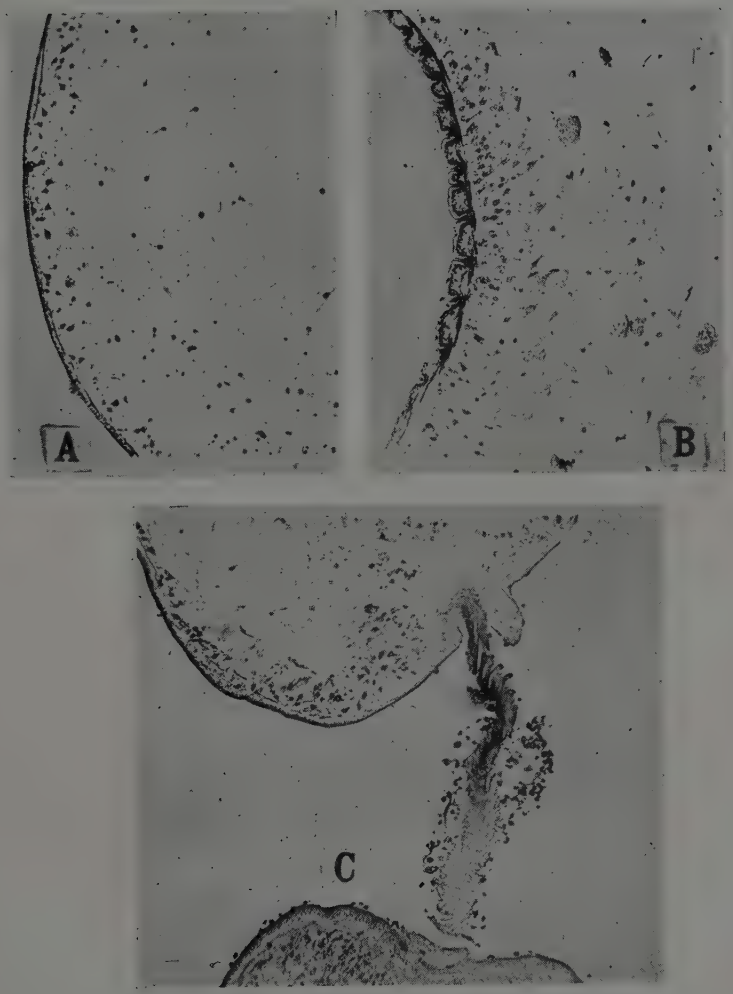


Plate I. Photomicrographs of portions of longitudinal sections through *C. pisiformis*. (X 300.) *A*. Normal bladder-wall of a cysticercus undergoing transverse fission. (Carnoy : Heidenhain's haematoxylin. 6μ thick.) *B*. Bladder-wall of same in region of annular constriction. The hypertrophied cuticle, muscle fibres and subcuticular cells are seen on the left, the granular masses released into the lumen, on the right. *C*. Portions of dividing cysticercus, joined by fibrous cord. Note the degenerating cells around cord, and the enlarged vacuolated cuticle at anterior and posterior ends of cord. (Carnoy : Chlorazol black E. 4μ thick.)

To face p. 178

Journal of Helminthology, Vol. XXII, Nos. 3/4, 1948, pp. 179—198.

*Further Studies on the Development of *Cysticercus fasciolaris* and *Cysticercus pisiformis*, with special reference to the Growth and Sclerotization of the Rostellar Hooks.

By Hilary Crusz, Ph.D., B.Sc.

(Assistant Lecturer in Zoology, University of Ceylon).
(From the Department of Parasitology, London School of Hygiene and Tropical Medicine.)

In a previous paper (Crusz, 1947) the writer gave a detailed account of the development of the rostellum and hooks of *Cysticercus fasciolaris* obtained from naturally infested rats in Colombo, Ceylon. There remained, however, the question of the time taken by this bladder-worm to arrive at various stages in its development : a point which could have been settled only by experimental infestations. Mlodzianowska (1931) gave details of the development up to the 15th day of infection, long before the appearance of the scolex-anlage, and Raum (1883) and Bartels (1902) in their experimental studies failed to observe the crucial stages in rostellar development that occur after the first appearance of the scolex-invagination. The mode of thickening of the hook-wall of taeniids and the exact relationship of the base of the hook to its blade were also points requiring elucidation by differential staining and histochemical methods.

A study of the larval development of *Taenia taeniaeformis* in experimentally infested hosts was therefore undertaken. A similar study of *Taenia pisiformis* was also made for checking previous accounts and for comparison with that of *T. taeniaeformis*. The results of these studies are set out in this communication.

Experimental Work.

Cysticercus fasciolaris.

Experiment I. Nine laboratory-bred rats (*Rattus rattus* : six-weeks-old males) were infected orally with half a gravid proglottis each of *Taenia taeniaeformis* freshly collected from the small intestine of a cat. Four of these rats died on the 4th, 10th, 15th and 25th day after infection. The rest were sacrificed, one on each day, on the 33rd, 35th, 40th, 47th and 60th day after infection. All the rats, except the first, showed numerous encysted bladderworms in the liver, and progressive

*Part of a thesis approved by the University of London for the award of the Ph.D. Degree.

development of these bladderworms was found to have occurred up to the 33rd day. Thereafter, degenerative changes seem to have set in, for the 35 to 60-days-old cysticerci were found to have been arrested in their development, as most of them were acephalic. The rat that died on the 4th day showed no traces of infestation in liver sections.

Experiment II. Ten laboratory-bred rats (five-weeks-old males) were similarly infected with fresh material from another cat. In this case all the rats were sacrificed on the 36th day and in the liver of one of them a larva was found which represented a stage later than that of the 33rd day larva of the previous experiment. In another of these rats a small cysticercus was found in which the bladder and scolex-anlage were definitely undergoing fibrotization: an indication of degenerative change. The other rats showed no signs of infestation apart from calcified patches in the liver.

From these two experiments it was possible to study the development of bladder and scolex, as the 10-, 15-, 25-, 33- and 36-days-old larvae provided a complete picture of larval development from the stage of the undifferentiated bladder right up to that of the fully differentiated scolex-invagination.

Cysticercus pisiformis.

The details of the experimental work which provided the material for a study of this form have already been described in a previous paper (Crusz, 1948c). It suffices to recall here that three laboratory-bred rabbits were infected orally with gravid proglottides of *Taenia pisiformis* obtained from a dog, and that all three rabbits showed very heavy infestation of liver, body-cavity and lungs, when autopsied on the 18th, 25th and 31st day after infection. Although the bladderworms taken from each of the three rabbits fell into three distinct groups from the point of view of size and state of development, there was so much overlapping of stages that even with 31-days-old larvae alone a complete study of larval development was possible.

Histological Study.

For histological study of the various stages of development, entire cysticerci or the scolex-anlagen alone, or, in the case of very early stages, pieces of infested liver, were washed in physiological salt solution and fixed in various fluids. Some of the material was fixed directly, without previous washing. Carnoy (6 :3 :1), Sansom-Carnoy, Helly, Petrunkewitsch, Bouin, formal-saline, 10% formalin and

absolute alcohol were the fixatives used. The fixed material was embedded in paraffin, and serial sections 4μ to 8μ thick were cut and stained in Heidenhain's iron haematoxylin with or without eosin, chlorazol black E and Mallory's triple stain. Carnoy, Helly and Bouin proved to be the best fixatives for histological work, while the material fixed in absolute alcohol and in formalin was indispensable for some of the histochemical tests. All the stains employed proved useful for various reasons: Heidenhain's haematoxylin for the finer details of structure, Mallory for marked selective staining of cuticle as distinct from the other structures like the primitive hooks, and chlorazol black E as a general stain of great merit.

Development of *Cysticercus fasciolaris*.

10 *days after infection.* (Figs. 1 and 7.)

The youngest larva studied is an elongate bladder measuring 0.31 mm. by 0.14 mm. Others of the same age are already more or less spherical (Fig. 1), and are from 0.20 mm. to 0.46 mm. in diameter. The bladder-wall is at this stage very rudimentary, as is shown by its cellular make-up. Investing the entire bladder is a delicate cuticle (cu) which is broken up peripherally into fine hair-like processes (hr). Below the cuticle and closely attached to it is the subcuticula (sc) in which the nuclei appear as large deeply staining vesicular structures scattered here and there, with other chromatoid material, in an irregularly disposed cytoplasm in which cell-boundaries cannot be distinguished. In some regions the cytoplasm forms a layer (basement membrane, bm) adjoining the cuticle, while in some it already shows signs of attenuation into the typical cytoplasmic processes of later stages. There can be no doubt that the cuticle is a secretion of this subcuticula and that it is being continually shed and renewed by the latter. The subcuticula is in such an undifferentiated condition in early stages that one cannot readily distinguish between "epithelial" cells, muscle-cells and parenchyma. The muscle fibres have not yet been formed. In cysticerci which are 0.2 mm. or more in diameter some very characteristic cells (mc) can be seen, which by their form and position appear to be primordial muscle-cells. They lie just below the cuticle and are seen to be distributed at some distance from each other all along the bladder-wall. They can be readily recognized by their hemispherical form, deeply staining cytoplasm and round nuclei. Their cytoplasm is drawn out into thin processes which run for some distance at least, just below the cuticle, and which are presumably the primordia

of the circular and longitudinal muscle fibres found in this region in older larvae. (Fig. 9, cm, lm.)

Within the bladder-wall is a relatively large bladder-cavity (bc) which in some specimens is still found to contain some of the cells which filled it in earlier stages. Mlodzianowska (1931) has observed that the bladder-wall is lined within by spherical nucleated masses of cytoplasm (Plasmakugeln) which project into the bladder-cavity, and he was unable to explain their presence. In some of the present material such masses are seen to have lost their shape and most of their nuclei, and are highly granular. They are undoubtedly metamorphosed cells, which appear to be involved in the formation of fibrous processes. The occurrence of such masses is not unknown wherever such "fibrotization" is active, as in the regions of transverse fission in *Cysticercus pisiformis* (Crusz, 1948c). Although some of the cysticerci at this stage appear elongate or ovoid, the writer has found it impossible to convince himself of the presence in them of "anterior" and "posterior" cell-aggregations. (Mlodzianowska, 1931.)

15 *days after infection.* (Fig. 8.)

The bladderworms have now assumed a spherical form, have grown larger (0.6 mm. to 1.0 mm. in diameter) and are distended with cystic

Abbreviations used in Figures 1–17.

b base of hook; **bc** bladder cavity; **bl**, inner, deeply staining layer of blade; **bl**$_2$ outer layer of blade; **bm** basement membrane; **bu** bulb; **bu'** posterior region of bulb; **cm** circular muscle fibres; **cu** cuticle; **cu'** modified cuticle filling cuticular folds; **em** eminence round prebulbar region; **hk** definitive hooklet; **hr** cuticular "hairs"; **lm** longitudinal muscle fibres; **mc** primordial muscle cell; **mf** musculo-fibrous limiting layer of bulb; **ml** muscle layers; **pb** prebulbar region; **pc** parenchyma cell; **pr** cytoplasmic process; **re** rostellar rudiment; **rec** receptaculum; **sa** scolex-anlage; **sc** subcuticula; **sk** sucker.

Development of Cysticercus fasciolaris.

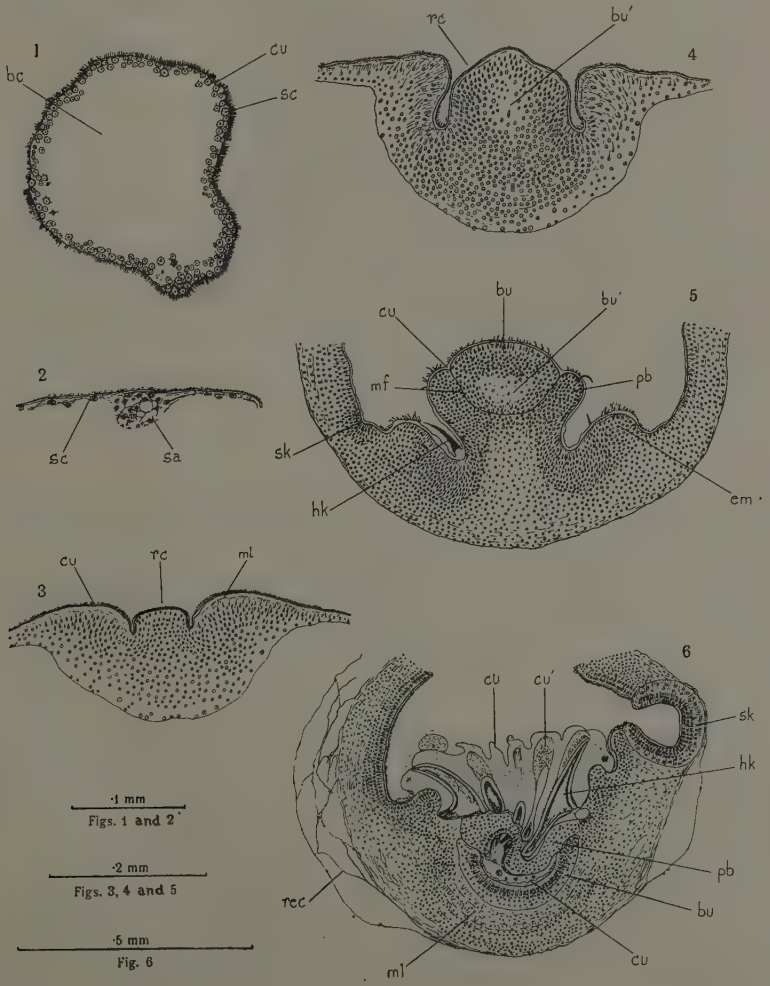
Fig. 1. Longitudinal section through entire 10-days-old bladder-worm.

Figs. 2, 3 & 4. Portions of median longitudinal sections through 25-days-old bladder-worms with scolex-anlagen at different stages of development.

Fig. 5. Median longitudinal section through 33-days-old scolex-anlage showing differentiation of rostellar rudiment into bulb and prebulbar region, and the rudiments of hooks and suckers.

Fig. 6. Median longitudinal section through 36-days-old scolex-anlage, showing rostellar rearrangements and further development of cuticle, hooks and suckers.

fluid. The subcuticular cells now tend to become differentiated into the subcuticula proper (sc) whose cells lie closed to the cuticle, being connected with a basement membrane by short strands of cytoplasm, and the parenchyma cells (pc) which lie farther away from the cuticle. All the cells appear stellate and there is cytoplasmic continuity between

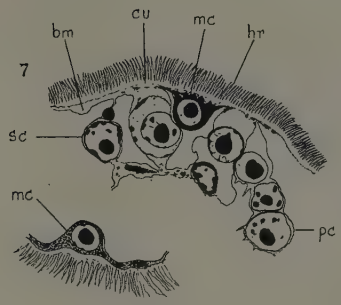


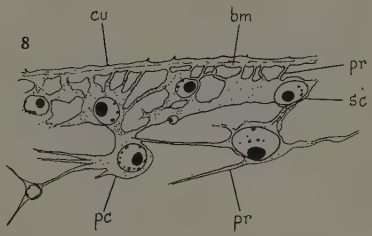
them as is shown by the anastomoses of their cytoplasmic processes (pr). The muscle-layers between cuticle and subcuticula are not yet fully formed. At any rate they are difficult to distinguish even with an oil-immersion lens. The primordial muscle-cells can, of course, still be clearly seen.

25 *days after infection.* (Figs. 2, 3 and 4.)

Bladderworms at this stage measure from 1.0 mm. to 1.7 mm. in diameter and are interesting for the fact that the scolex-anlage has appeared in most of them. It is also interesting to note that the scolex-anlagen are not all at the same stage of development. At least three distinct stages are to be seen. The scolex-anlage develops as a local hemispherical thickening of the bladder-wall, brought about by cell-proliferation. It is from 0.03 mm. to 0.05 mm. long and 0.07 mm. to 0.10 mm. broad (Fig. 2). In one bladder-worm two such scolex-anlagen were found to develop some distance from each other : this establishes for certain the fact that polycephalous cysticerci are the result of such asexual proliferation from the bladder-wall at an early stage in its development.

A scolex-anlage of a later stage is shown in Fig. 3. It is 0.16 mm. long and 0.34 mm. broad. Further cell-proliferation has resulted in the formation of a characteristic hemispherical structure in which the rudiment of the rostellar cone has already been laid down (rc). Scolex-anlagen of a still later stage (Fig. 4) are present in the majority of the 25-days-old cysticerci. Here the rostellar rudiment is assuming its characteristic shape, having grown upwards from the bottom of the invagination cavity. The histology of all these stages is precisely the same as that described before (Crusz, 1947), but a few points are to be specially noted. A close study of the developmental stages depicted in Figs. 2, 3 and 4 would seem to indicate that there are, in the earliest undifferentiated hemispherical scolex-anlage, definite cell-regions which give rise to specific structures in later stages. One such region (Fig. 3, rc) was also figured by Gläser (1909, Fig. 2) for *Cysticercus longicollis,* and must represent the rudiment of the rostellar bulb (bu). Another interesting point is that arising from the next stage (Fig. 4). There is in the centre of the rostellar cone a region (bu') where cells are fewer in number as compared to surrounding regions. Such a paucity of cells





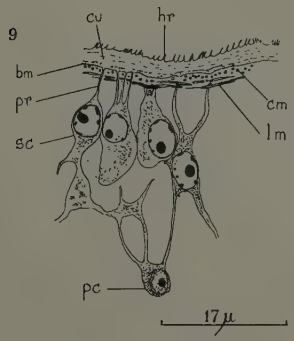


Fig. 7. Portion of a section through 10-days-old bladder-worm (Fig. 1), showing histology of bladder-wall. The cells shown make up the entire thickness of the bladder-wall in this region.

Fig. 8. Portion of a section through 15-days-old bladder-worm.

Fig. 9. Portion of a section through 36-days-old bladder-worm. Only the cuticle and part of subcuticular region are shown.

exists in the posterior region of the bulb of the 33-days-old larva (Fig. 5, bu'), so that it is possible to refer to the anterior and posterior regions of the future bulb in as early a stage as that represented by Fig. 4. This paucity and more or less vertical orientation of cells is correlated with the formation of vertical (radial) muscle fibres in that region of the bulb. It is a constant feature of all the material studied both of *C. fasciolaris* and *C. pisiformis*.

If one now compares the three stages described above with the stages figured previously (Crusz 1947, Figs. 1 and 2) one finds that in the latter the scolex-anlagen are much longer and the rostellar rudiments have been carried farther into the bladder-cavity : a fact which indicates that in the early stages the region of most active cell-proliferation is that portion of the bladder-wall immediately surrounding the scolex-anlage (Gläser, 1909).

33 *days after infection.* (Fig. 5.)

This stage of development was discovered in interesting circumstances. The liver of the rat sacrificed 33 days after infection showed only about a dozen encysted cysticerci all of which proved to be acephalic. The material was fixed as usual and laid aside. On re-examining the material the liver was macerated and was found to contain an encysted bladder-worm in which the scolex-anlage was well-formed and at a later stage of development. This scolex-anlage is 1.0 mm. long and 0.7 mm. broad. The rostellar rudiment, 0.17 mm. long and 0.24 mm. broad, is differentiated into bulb (bu) and prebulbar region (pb) both of which carry numerous spines or hooklets. The posterior region of the bulb shows clearly the less compact arrangement of cells (bu'), already referred to. At the bottom of the furrow surrounding the prebulbar region are the definitive hooklets (hk) formed on conical extensions of the cuticle. These hooklets are from 51μ to 58μ long. Beyond this furrow is the mound-like eminence (em) which is destined to produce the hypertrophied cuticle that surrounds the thickening definitive hooks, and beyond this eminence are four shallow depressions involving cuticle and subcuticula : the rudiments of the suckers (sk). Minute hooklets are also to be seen all along the cuticle beyond the region of the definitive hooklets and their distribution stops short of the suckers. A comparison of Fig. 5 with the photomicrograph published earlier (Fig. 10, Crusz 1947) shows that the stages of development described in both cases are almost identical. One point, however, needs clarification. A study of all the material available, both of *C. fasciolaris*

and *C. pisiformis,* has convinced the writer that there are two distinct elements separating bulb from prebulbar region, namely the cuticle (cu) which runs only for a short distance between the two regions but never completely separates them, and a fine deeply staining musculo-fibrous sheet (mf) which separates the two regions completely. In fact this layer of fibres is distinctly seen to be continuous with the subcuticular muscle-fibres of bulb and prebulbar region. On this point the histology of the fully differentiated rostellar rudiment of cysticerci seems to be in complete agreement with Goldschmidt's description (1900) for *Echinococcus,* rather than with Gläser's (1909) for *Cysticercus longicollis.* Gläser regards this separation to be effected by a complete layer of cuticle.

36 *days after infection.* (Figs. 6 and 9.)

At this stage, which represents the final phase in the differentiation of the bladder and invaginated scolex, the prebulbar region (pb) has closed over the bulb which has sunk completely and lies fully extended (bu). The hypertrophy of the cuticle (cu) in the region involved in hook-development is very striking. The entire cuticle between suckers and prebulbar region has become greatly enlarged, is deeply folded and lies in close contact with the hook-blades (hk). It is highly modified in that it appears granular and vacuolated and also contains a few round vesicles filled with granular substance (Gläser 1909, Crusz 1947). The cuticular folds (cu') in which the hook-blades lie are also filled with granules. The hooks themselves have thickened considerably and their bases have also been just formed. Each of the four cup-shaped suckers (sk) has its full complement of cuticle, subcuticular cells and muscle-fibres, of which the vertical (radial) muscle-fibres are the most prominent. Several muscle-layers (ml) are also present below the sunken bulb. The outermost layers of the entire scolex-invagination are now definitely fibrous and constitute the fibrous receptaculum (rec) which in other species is developed almost as soon as the scolex-anlage appears.

The bladder of this specimen now reveals all the typical structures such as cuticle, basement membrane, muscle-layers, subcuticula and parenchyma. A portion of the cuticle and subcuticula is shown in Fig. 9. The subcuticular cells (sc) now lie perpendicular to the cuticle and are connected with the basement membrane by fine cytoplasmic processes (pr). The parenchyma cells (pc) are distinctly in connection with the subcuticular cells by similar cytoplasmic processes. This alignment of

the subcuticular cells perpendicular to the cuticle and the development of the circular and longitudinal muscle-fibres actually start in the scolex-anlage at the time it begins to invaginate, and later on become a general feature of the entire bladder-wall. The parenchyma becomes very extensive and the excretory system is well developed at this stage as is shown by the numerous flame-cells and excretory ducts in section. Excretory ducts are in fact to be seen in the scolex-anlage and bladder-wall in earlier stages.

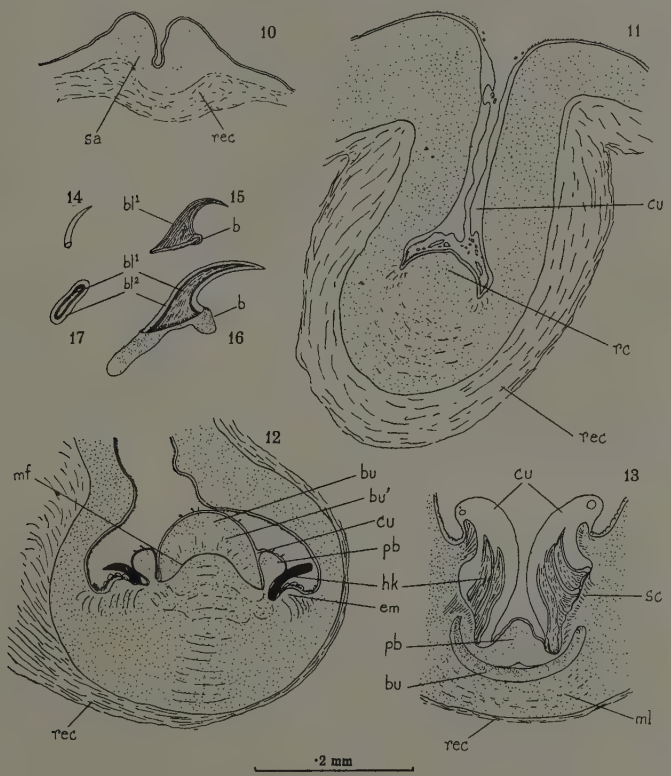
The above study while confirming and extending the previous work (Crusz, 1947) shows that not only the hemispherical scolex-anlagen (Raum, 1883) but also older scolex-anlagen in which the rostellar rudiment has been just laid down or even differentiated further, can be met with in 25-days-old bladderworms of this species. The rostellar rudiment becomes differentiated into bulb and prebulbar region by the 33rd day and these take up their final positions by the 36th day. There can be no doubt that the developmental process is subject to much variation due to factors governed by the host-parasite relationship, but it seems to be generally true from this and previous work that the full development of the various parts of the invaginated scolex is not achieved until well over a month from the time of ingestion of eggs by the host.

Development of *Cysticercus pisiformis*.

Our knowledge of the development of this species is due chiefly to the researches of Leuckart (1856, 1886), and to Young (1908) who made a detailed study of its histogenesis. A study of the present material, most of the details of which were described earlier (Crusz, 1948c), has resulted in ample confirmation of their main findings, so that it suffices to draw attention here only to a few points on which their accounts are not sufficiently clear, and also to compare the development of this bladderworm with that of *C. fasciolaris*.

The scolex-anlage (sa) and rostellar cone (rc) of *C. pisiformis* are formed during the third week of infestation (16-18 days) (see Figs. 10-13). The bulb (bu) and prebulbar region (pb) of the rostellum are differentiated during the fourth week (23-25 days). The histology of these regions corresponds very closely to that described for *C. fasciolaris*. In the anterior region of the young bulb (Fig. 12, bu) and below its cuticle, the cells are closely packed and form a broad band of tissue. The cells of the posterior region of the bulb (bu′) are fewer in number and loosely arranged. They are vertically orientated and their nuclei are

ovoid and their cytoplasm drawn out into anterior and posterior processes which are the forerunners of the vertical (radial) muscle fibres found in this region in the mature bulb. Separating the bulb from the



Development of Cysticercus pisiformis.

Figs. 10 & 11. Median longitudinal sections through scolex-anlagen in third week of development.

Fig. 12. Median longitudinal section through scolex-anlage in fourth week of development.

Fig. 13. Portion of median longitudinal section through 31-days-old scolex-anlage.

Figs. 14, 15 & 16. Definitive hooks at three stages of development.

Fig. 17. Transverse section through blade of mature hook as seen in stained sections of scolex.

prebulbar region and from the underlying tissues is a distinct layer (mf) of muscle fibres which are continuous with the subcuticular muscle fibres of bulb, prebulbar region and the region beyond. The cuticle (cu) is also seen to separate these two regions, but as in *C. fasciolaris,* only incompletely. Hooklets or spines are to be found as usual over the entire cuticle of the rostellum up to the suckers. Most of them drop off early, but the definitive hooklets grow out in their usual position at the bottom of the furrow around the prebulbar region. The eminence (em) beyond the region of the definitive hooklets is also a distinctive feature at this stage. It develops an extraordinarily hypertrophied cuticle in later stages. The last stage in the differentiation of the various parts of the rostellum is shown in Fig. 13, where the prebulbar region has now completely closed over the sunken bulb and where the thickening hooklets and hypertrophied cuticle assume their characteristic appearance. Most of the 31-days-old larvae are at this stage of development.

A comparison of the larval development of *C. fasciolaris* with that of *C. pisiformis* shows some easily recognizable differences between them. The development of *C. pisiformis* is relatively faster in that the scolex-anlage appears during the third week of infestation or even earlier and is generally fully differentiated in about a month's time. The scolex-anlage of *C. fasciolaris* appears during the fourth week of infestation and takes well over a month to be fully differentiated. *C. pisiformis* is an elongate bladder-worm, and not spherical like *C. fasciolaris,* right up to the last stage of rostellar development, and its central bladder-cavity enlarges much more slowly than that of *C. fasciolaris.* In fact, a comparison of a 10-days-old *C. fasciolaris* with an 18-days-old *C. pisiformis* shows that while in the former the bladder-cavity is almost fully formed and contains hardly any central parenchymatous tissue, the latter has a relatively large amount of this tissue. At the same time one observes a striking development of the subcuticular muscle layers in *C. pisiformis* from its earliest stages, whereas in *C. fasciolaris* they are feebly developed and almost imperceptible at corresponding stages. This is obviously correlated with the great powers of movement of *C. pisiformis* and its early migration from the liver into the body-cavity.

It is also evident that the main differences between the two species, besides differences in size, are those concerned with the above-mentioned peculiarities in the development of the bladder. The scolex-anlage, although it appears much earlier in *C. pisiformis,* develops on the same

lines as that of *C. fasciolaris*. It is much smaller, but there is marked correspondence in histological details between the two species. There are however two striking differences. In *C. pisiformis* the receptaculum (rec) is well developed. It is largely formed of fibres developed from the parenchymatous reticulum adjoining the early scolex-anlage and is carried into the bladder-cavity and developed further as the scolex-anlage invaginates and grows in size. In *C. fasciolaris* this receptaculum is not at all evident in the early developmental stages. When the rostellum and hooks have been developed, however, a fibrous sheath appears all round the invaginated scolex. It is quite clear that the difference between the two species in this regard is not merely a question of time, in that the receptaculum of *C. fasciolaris* is formed very late in its development, but is also a question of origin. In *C. fasciolaris* it is formed by the differentiation of the outer-most cell-layers of the definitive scolex-anlage. In *C. pisiformis* it is formed chiefly from the relatively undifferentiated parenchymatous reticulum adjoining the scolex-anlage in the early stages. The other marked difference between these two forms is that the characteristic flexure of the scolex-invagination takes place very early in *C. pisiformis*, certainly before the differentiation of the rostellar cone into bulb and prebulbar region. In *C. fasciolaris* it occurs long after this differentiation takes place.

In regard to the development of the rostellum and suckers and of the primitive hooklets and definitive hooks, there is substantial agreement between the two species. A close comparison of the details described here for *C. fasciolaris* and *C. pisiformis* with Gläser's account of the development of *C. longicollis*, shows clearly that the development of the rostellum, suckers and hooks of these three species of cysticercus are essentially the same and that any differences noted, as for instance in the range of distribution of the primitive hooklets or spines, are not valid differences, for the entire cuticle up to the suckers is covered at some time or other with these structures and the distribution noted just depends on how many of them have fallen off at the particular stage observed.

Growth and Sclerotization of Rostellar Hooks.

The writer has already given a detailed account (Crusz, 1947) of the development of the rostellar hooks of *Cysticercus fasciolaris* and has presented some preliminary facts about their chemical nature. It was

thought to be interesting and useful to make a closer study of the problem of hook development with the aid of differential staining and histochemical tests. The material used in this study came from larvae and adults of *Taenia taeniaeformis, T. pisiformis, T. hydatigena* and *T. serialis*. The hooks of these forms were found to be so similar in their staining properties and reactions to the various tests, that a common description, applicable to all of them, could be given here. It must be mentioned, however, that, in regard to the developmental stages before the formation of the base of the hook, the material came only from *T. taeniaeformis* and *T. pisiformis*. The results obtained are summarized in Table I. A brief discussion is given below, which is followed by a résumé of all the facts now known in regard to the histo- and chemo-differentiation of the taeniid hook.

Stains : The staining reactions of hooks were observed for the most part in the course of the histological study of sections. The hooklets (Figs. 14 and 15; see also Crusz, 1947, Figs. 6, 10, 11 and 12) stain intensely with haematoxylin, Mallory and chlorazol black E, at all stages of development, and they always stain differently from the cuticle as is best shown in Mallory-stained preparations, where the cuticle stains blue and the hooklets orange. In the mature blade (Figs. 16 and 17), on the other hand, there is an inner "membrane" (bl_1) which stains intensely, while the rest (bl_2) is refractory to staining. The base (b) is also refractory to most stains, but Mallory colours it a deep orange. Picric acid stains the entire hook very intensely.

Tests for Protein and Sulphur: Various well-known tests were performed on hooks freshly obtained and washed in several changes of distilled water; on hooks from material preserved in alcohol or formalin; on those obtained from the peptic and tryptic digests of scolices; and on sections of alcohol- or formalin-fixed scolices and scolex-anlagen. The results were similar in all cases and show the base of the hook to be composed of a protein which reacts positively to all these tests, while the blade in its early stages (Figs. 14 and 15) shows definite positive reactions to some of the protein colour-tests. All except the xantho-proteic and ninhydrin reactions seem to fall off in intensity as the blade thickens and hardens. Sulphur is undoubtedly present in the entire hook as is shown by the dense precipitation of black lead sulphide, accompanied by swelling and disintegration, when hooks are treated with sodium plumbite solution in the cold. The remarkable swelling and gradual disintegration of the mature hook in 2% sodium sulphide

solution (with or without alkali) seem to indicate the presence of -S-S- linkages as in keratin. The soft hooklets are unaffected by sodium sulphide but turn brown when heated with sodium plumbite. It is interesting to note in this connection that in their studies on the chemical composition of whole tapeworms Smorodintzev and Pavlova (1936) obtained a sulphur-bearing fraction which they designate

TABLE I.

	Hooklet (Figs. 14 & 15)	*Hook* (Fig. 16)		
		blade.		*base.*
		bl_1 (inner)	bl_2 (outer)	b
Stains.				
Haematoxylin	+++	++	—	—
Mallory	+++	++	—	+++
Chlorazol black E.	+++	+	—	—
Picric acid	+++	+++		+++
Protein colour-tests.				
Biuret (for peptide linkage) ..	..	+		+++
Ninhydrin (for α-amino-acids)	+	++		+++
Xanthoproteic (for benzene ring)	+	+++		+++
Millon's (for phenol radical) ..	++	+		+++
Sakaguchi's (for arginine) ..	..	+		+++
Sulphur tests.				
Sodium plumbite	+	+++		+++
Sodium sulphide (for -S-S-linkage)	—	+++		+++
Polyphenol tests.				
Argentaffin (for reducing agent)	++	+++	+	+
Chromaffin	—	—	—	—
Potassium iodate	—	—	—	—
Chitin tests				
Chitosan test	..	+?		—
Schulze's test	—	++		—
Peptic digestion	..	—	—	—
Tryptic digestion	..	—	—	—
Polarized light	Birefringent	Birefringent		Isotropic

"keratin". Similarly, Salisbury and Anderson (1939) report a 0.20% cysteine content for the strobilocercus (*Cysticercus fasciolaris*) of *Taenia taeniaeformis*.

Tests for polyphenols: These tests, described by Lison (1936), and used extensively in recent years for the study of insect material (Pryor 1940, Dennell 1946) and trematode material (Stephenson, 1947), were performed on sections. Hooklets at the stage represented by Fig. 15, and older ones, are distinctly blackened by ammoniacal silver hydroxide (argentaffin test). In the mature hooks the inner reaches of the blade react very intensely, while the rest assumes a brown coloration. The minute hooklets do not give the reaction, nor do the cuticle and the other tissues of the scolex. The chromaffin and potassium iodate tests are negative. The positive argentaffin test indicates the presence of a sufficiently strong reducing agent in the hook-substance and is strongly suggestive of polyphenols. However, there is no appreciable darkening of hooks as they mature, as one encounters in phenolic hardening.

Tests for Chitin: Schulze's test for chitin (chloro-zinc-iodine after diaphanol) gives a distinct violet coloration of the blade of the mature hook, while the base is clearly unaffected by this treatment. This indicates the presence of chitin in the blade alone. Campbell's chitosan test which was previously (Crusz, 1947) reported to be negative, was therefore repeated several times with variations in technique. A particularly useful modification was found to be the postponement of the examination of any residual matter left by the superheating, until after the treatment with iodine – H_2SO_4. In this way a few small soft, purplish cones were seen, which appeared to be the remains of hook-blades. These dissolved in concentrated H_2SO_4. The bases of the hooks were, of course, nowhere to be seen. The great difficulty experienced in the application of this test to the present material, especially in the matter of detecting the residual elements, leaves no doubt as to the fact that the greater portion of the taeniid hook is non-chitinous, but the definite result obtained with Schulze's test, and the observations made above, point to the presence of chitin in the blade of the hook. The young hooklets do not react to Schulze's test.

Peptic and tryptic digests: For the purpose of noting the effect of digestive juices on the hook, entire bladderworms (*Cysticercus tenuicollis*) or mature scolices only (*C. fasciolaris*) were macerated and subjected to the technique used by Campbell (1942). The material was defatted by refluxing successively with ethanol, ether and benzene in a Soxhlet apparatus, and water-soluble matter was extracted at pH 4.5 and later at pH 8.5. The material was then subjected to peptic digestion at pH 1.6 and later to tryptic digestion at pH 8.0, both at

a temperature of 38° C. The rostellar hooks were found to be entirely resistant to hydrolysis by these digestive enzymes, and were centrifugally separated and washed in several changes of distilled water. Protein and sulphur tests performed on this material gave the same results as before. It should be mentioned here that taeniid hooks are also remarkably resistant to prolonged treatment with dilute and concentrated HCl and H_2SO_4.

Polarized light : The results obtained with polarized light confirm in a striking manner the differences hitherto noted between the blade and base of the taeniid hook. The blade is a distinctly birefringent structure (Figs. 18-21) when viewed as a whole and in section, and is so at all stages of its development. Immersion in fluids of varying refractive index (1.33 to 1.66) (Picken 1940, Pantin 1948) shows no appreciable change in its birefringence, which is therefore largely of an intrinsic character. This is significant because orientated keratinous and chitinous structures show both intrinsic and form birefringence although in most structures the latter is the more important. It is interesting to note that, as in the keratin of human finger-nail, the birefringence of the blade disappears gradually on treatment with sodium sulphide solutions, probably due to the breakdown of the parallel arrangement of protein chains.

The base of the hook is isotropic in polarized light, so that there obviously exists a structural difference between the proteins of base and blade. This seems to be correlated with the fact that while the blade is remarkably constant in shape and proportions within a species, the base is more variable and is often the seat of most of the anomalies in hook-structure.

Cuticle : Very little information was obtainable on the question of the chemistry of the developing cuticle, due mainly to very real difficulty in accurate observation and to the intimate manner in which the cuticle is bound up with subcuticular structures. However, it is noteworthy that the hooks at all stages react differently from the larval cuticle in the way in which they stain with haematoxylin, Mallory and chlorazol black E, and that, unlike the hooks, the cuticle yields to peptic and tryptic digestion. The cuticle gives positive xanthoproteic and Millon's reactions and a negative argentaffin reaction, and is isotropic when viewed in polarized light. Nevertheless it is intimately bound up with the development of hooks as histological studies clearly reveal, so that the development of blade and base and their hardening should involve

chemical reactions, probably of the oxidative type, between substances secreted at the foci of hook-development. It is also a very significant fact that the hypertrophied cuticle, which envelops the thickening blades, belongs mainly to a region of the scolex-rudiment different from that in which the blades were first developed, suggesting that it performs a different function, namely the hardening and orientation of the blades.

Conclusions.

From the foregoing account and from the previous work it is now possible to give a more detailed account of the differentiation of the rostellar hooks of taeniids. There are three distinct processes involved: the development of the blade (Figs. 14 and 15, bl_1), the thickening and hardening of the blade (Figs. 16 and 17, bl_1 and bl_2) and the development of the base, that is, the guard and handle (Figs. 15 and 16, b).

The blade of the hook originates from a small conical elevation of the cuticle, formed of fused cuticular "hairs", which secretes a soft, proteinaceous, birefringent structure (the hooklet or "spine"), staining differently from the cuticle and chemically and optically different from it. The bulb, prebulbar region and the region beyond are covered with such "spines" in early stages of development. Most of them grow no further and fall off, while those situated at the bottom of the furrow round the prebulbar region grow out into hollow, claw-shaped definitive hooklets by continued secretion of hook-substance from their respective cuticular cones. Each definitive hooklet grows broader at its base as the area of secretive activity increases with the growth of the scolex-anlage. Fig. 15 represents the last stage in the development of the soft, deeply staining blade which now appears longitudinally striated with an apparent increase in its birefringence. Thereafter two processes occur almost simultaneously: the blade thickens and becomes more brittle, and a hard base is deposited. Leuckart held that the blade thickens by deposition on its inner surface, while Gläser maintained that the deposition is on the outer surface. There are three points in favour of Gläser's view. The innermost lining of the mature hook stains distinctly, like the primitive hooklets, with haematoxylin, Mallory and chlorazol black, while the outer brittle layer, which is thicker, is refractory to staining. This inner "membrane" of the blade corresponds very closely in size and appearance to the hooklet which came before (compare Figs. 15 and 16). Lastly, the fact that at the time of hook-thickening the hypertrophied and structurally altered cuticle is in close contact with hook-blades at all points, strongly

suggests that it has at least some chemical connection with the thickening and hardening of the blades. The blade now shows distinct evidence of the presence in it of chitin in conjunction with a sulphur-bearing scleroprotein. It is birefringent and is attacked by sodium sulphide solutions.

The formation of the base begins as a horse-shoe-shaped secretion (Fig. 15, b) in the region of the future guard. It soon extends all round the bottom of the blade and ultimately forms a complete base which occludes the entrance to the hollow of the blade. By further secretion, the anterior and posterior extensions (handle and guard) of the base are formed. The base, at all stages of its development, is a thoroughly protein structure, is isotropic in polarized light, is non-chitinous, contains sulphur and is attacked by sodium sulphide solutions. That it is firmly associated with the blade to form a rigid structure is an unquestionable fact, but it is at the same time composed of a very definite type of scleroprotein and is chemically and optically different from the blade, which seems to account for the fact that it is more liable than the latter to variations in shape and size.

Summary.

The previous observations of the author and others on the development of *Cysticercus fasciolaris* and *C. pisiformis* have been confirmed and amplified by experimental studies, and the developmental processes of the two forms are compared. While definite information has been obtained on the age of the various developmental stages, there is considerable variation due to the subtleties of the host-parasite relationship.

The study of the development of the rostellar hooks of taeniids has been extended by the use of differential staining, histochemical tests and polarized light, and new facts, such as the chemical and optical differences existing between cuticle and hooklets and between blade and base of hooks, have been brought to light.

Acknowledgments.

The writer is very grateful to Professor J. J. C. Buckley for kind help and advice given throughout this work, and to Professor R. T. Leiper, C.M.G., F.R.S., for assistance with the literature, at the Commonwealth Bureau of Agricultural Parasitology.

He is also indebted to Dr. M. G. M. Pryor and Miss C. H. Brown of Cambridge University for their kind criticism and helpful suggestions in regard to the histochemical work, and to Mr. B. J. P. Alles of the

University of Ceylon, who assisted in the digestion experiments which were carried out there.

Mr. B. K. Johnson of Imperial College, London, and Dr. R. Barer of the Department of Human Anatomy, Oxford, very kindly helped the writer in the examination of material in polarized light. Dr. Barer is responsible for the photographs published herewith.

References.

Bartels, E., 1902.—" *Cysticercus fasciolaris*. Anatomie, Beiträge zur Entwicklung und Umwandlung in *Taenia crassicollis*." *Zool. Jber.*, Anatom., XVI, 511–570.

Braun, M., 1894–1900.—Vermes. Abt. Ib. Cestodes : in Bronn's Klassen u. Ordnungen des Thier-Reichs. Vierter Bd. *Leipzig*.

Campbell, D. H., 1942.—" Experimental eosinophilia with keratin from *Ascaris suum* and other sources." *J. Infectious Diseases*, LXXI, 270–276.

Crusz, H., 1947.—" The early development of the rostellum of *Cysticercus fasciolaris* Rud., and the chemical nature of its hooks." *J. Parasit.*, XXXIII, 87–98.

1948c.—" On the transverse fission of *Cysticercus pisiformis* in experimentally infested rabbits, and the phylogenetic significance of asexual phenomena in cysticerci." *J. Helminth.*, XXII.

Dennell, R., 1946.—" A study of an insect cuticle : the larval cuticle of *Sarcophaga falculata* Pand. (Diptera)." *Proc. Roy. Soc.* Ser. B., CXXXIII, 348–373.

Gläser, H., 1909.—" Zur Entwicklungsgeschichte des *Cysticercus longicollis* Rud." *Z. wiss. Zool.*, XCII, 540–561.

Goldschmidt, R., 1900.—" Zur Entwicklungsgeschichte der Echinococcusköpfchen." *Zool. Jber.*, Anatom., XIII, 467–494.

Leuckart, R., 1856.—Die Blasenbandwürmer und ihre Entwickelung. *Giessen*.

1886.—The parasites of man, and the diseases which proceed from them. Trans. by W. Hoyle. *Edinburgh*.

Lison, L., 1936.—Histochimie Animale. *Paris*.

Mlodzianowska, B., 1931.—" Über die jüngsten Entwicklungsstadien von *Cysticercus fasciolaris* Rud., der Larve von *Taenia taeniaeformis* Bloch, auf Grund von Experimentaluntersuchungen." *Bull. Internat. Acad. Polon. (Ser. B.) Sci. Nat.* II. Zool., 3/5, 475–511.

Pantin, C. F. A., 1948.—Notes on microscopical technique for zoologists. Second impression. *Cambridge*.

Picken, L. E. R., 1940.—" The fine structure of biological systems." *Biol. Rev.*, XV, 133–167.

Pryor, M. G. M., 1940a.—" On the hardening of the ootheca of *Blatta orientalis*." *Proc. Roy. Soc*, Ser. B, CXXVIII, 378–393.

1940b.—" On the hardening of the cuticle of insects." *Ibid.*, 393–407.

Raum, J., 1883.—" Beiträge zur Entwickelungsgeschichte der Cysticercen." Inaug. Dissert. 45. pp. *Dorpat*. (Figures in Bronn's Klassen u. Ordnungen. IV. Vermes.)

Salisbury, L. F. & Anderson, R. J., 1939.—" Concerning the chemical composition of *Cysticercus fasciolaris*." *J. biol. Chem.*, CXXIX, 505–517.

Smorodintzev, I. A. & Pavlova, P. I., 1936.—" Répartition de l'azote des fractions albumineuses dans le corps des Ténias." *Ann. Parasit. hum. comp.*, XIV, 489-494.

Stephenson, W., 1947.—" Physiological and histochemical observations on the adult liver fluke, *Fasciola hepatica* L. III. Egg-shell formation." *Parasitology*, XXXVIII, 128–139.

Young, R. T., 1908.—" On the histogenesis of *Cysticercus pisiformis*." *Zool. Jber.* Anatom., XXVI, 183–254.

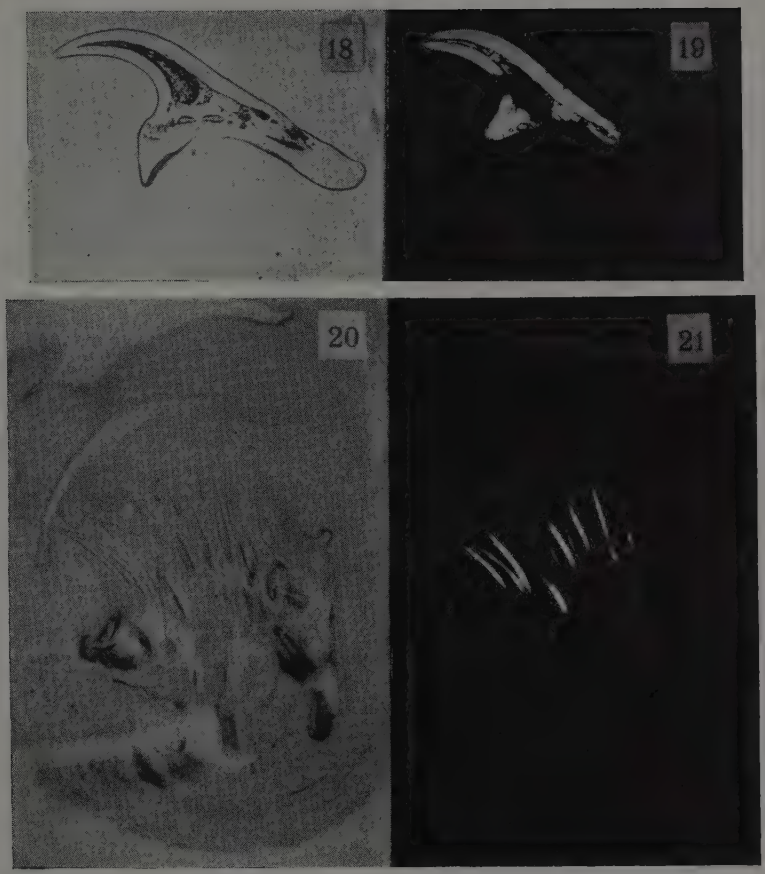


Fig. 18. Large hook of mature *Cysticercus fasciolaris*, photographed in ordinary light. (×150).

Fig. 19. Same hook in polarized light. Note that the blade alone, which is birefringent, has shown up.

Fig. 20. Longitudinal section through invaginated scolex of *C. fasciolaris*, photographed in ordinary light. (×100).

Fig. 21. Same field in polarized light, showing the birefringent hook-blades

To face p. 198

Journal of Helminthology, Vol. XXII, Nos. 3/4, 1948. pp. 199—218.

Studies on the Helminth Parasites of Birds in Hyderabad State.

Nematoda II.

By Satya Narayan Singh, M.Sc.,
Reader-in-charge in Zoology, Osmania University, Hyderabad Deccan.
(From the Department of Parasitology, London School of Hygiene and Tropical Medicine.)

Acuaria Bremser, 1811.

Acuaria anthuris (Rudolphi, 1819) Railliet, Henry and Sisoff, 1912.
Syn. *Acuaria scutata* Maplestone, 1931.

This species was collected on several occasions from the common house-crow, *Corvus splendens*, and the jungle-crow, *C. macrorhynchus* in Hyderabad. As earlier descriptions of this species were inadequate, the writer thought it advisable to give a detailed account of the worm which happens to be the type of the genus *Acuaria*.

The worms occur under the horny lining of the gizzard, the females being considerably larger and stouter than the males. The body in both sexes is of almost uniform diameter and is attenuated only for a short distance near the head and tail ends. The cuticle is thick and transversely striated. The cordons are well developed; they start in pairs from the angles of the mouth in a dorso-ventral position, and diverge as they run backwards beyond the oesophagus and, as a rule, they are proportionately longer in the females than in the males. They are in a double line for most of their length but at the posterior end they are in the form of a single band. In addition to the true cordons there are also present "pseudo-cordons" which lie in the lateral fields. They extend along the entire length of the body starting in the region of the pharynx where they are faintly developed, being nothing more than mere interruptions of the cuticular striae. Further back they gradually become more prominent and have a characteristic shape as shown in Figure 10. They continue posteriorly to the tip of the tail. The cervical papillae are very minute and are at a distance of 308μ from the head-end in a female measuring 18.9 mm. in length. The nerve-ring encircles the muscular portion of the oesophagus near its origin. The head bears two pairs of well developed cephalic papillae and also a pair of "amphids" or lateral papillae. The end-on view (Figure 2) closely corresponds with that given by Chitwood and Wehr (1934) for *Acuaria anthuris*. The mouth leads into a cylindrical pharynx whose

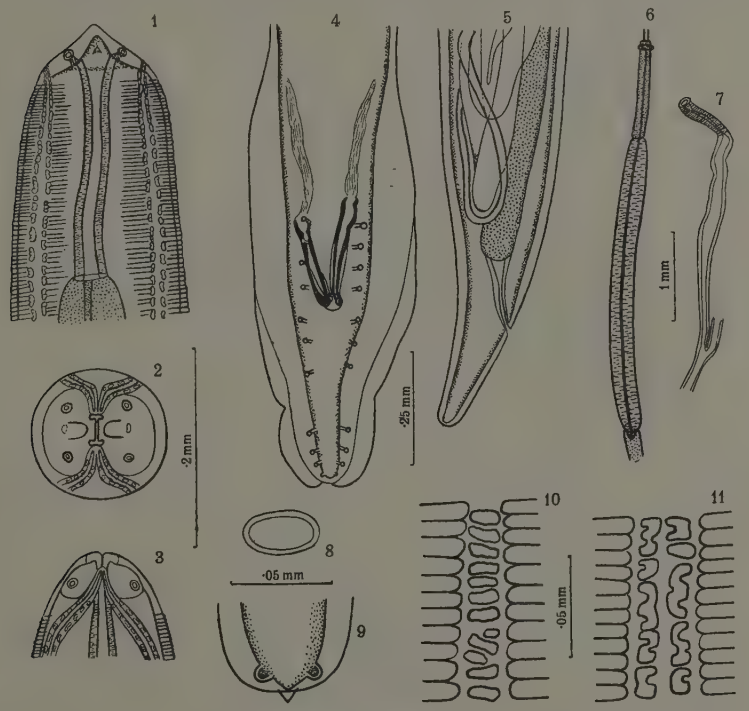
anterior end presents a funnel-shaped appearance in lateral view. It is followed by an oesophagus which is differentiated into a short anterior muscular portion and a long glandular posterior portion. The intestine runs in a straight course to the posterior extremity where it communicates with the rectum.

Female: The female measures 18.9 to 27.5 mm. in length with a maximum diameter of 414 to 450μ. The cuticular striations are 10.3-11.6μ apart. The cordons extend 7.128-7.2 mm. from the anterior end. The pharynx is 210 to 233μ long and the two portions of the oesophagus measure 1.1. to 1.619 and 3.34 to 4.618 mm. respectively. The tail is very short, and bluntly rounded at the tip. It is about 256μ long and bears a pair of subterminal papillae. It is surmounted by a spike-like projection at the tip. The vulval opening is situated at about the middle of the body and may be slightly anterior or posterior to it. In a specimen measuring 18.9 mm. long it was 8.428 mm. and in another specimen measuring 25.75 mm. it was 13.5 mm. from the head end. The vagina at first forms a forwardly directed loop and then joins the posteriorly directed common trunk of the uterus, the two portions measuring .5-.747 and 2.4-2.5 mm. respectively. The uteri are opposed. The eggs are thick shelled and measure 38-42μ long by 22-24μ wide; they are embryonated at the time of laying.

Male: The male is smaller and more slender than the female, measuring 8.5-11.25 mm. in length and .256-.308 mm. in maximum transverse diameter. The cuticular striations in the middle of the body are 5.6-7.7μ apart. The pharynx is 210-214μ long; the two constituent portions of the oesophagus are .58-.642 and 1.78-2.1 mm. long respectively, the total length of the oesophagus varying from 2.36-2.722 mm. The cordons extend beyond the oesophagus and are 3.58-4.2 mm. long. The cloacal aperture is 400-420μ from the tip of the tail. The caudal alae are well developed and are about .9-.97 mm. long. The caudal papillae are pedunculated and there are altogether 11 pairs, out of which 4 pairs are pre-anal and 7 pairs post-anal in position. The latter are arranged in two groups of 3 and 4. The spicules are unequal and measure 209-219 and 251-262μ respectively. The body of each spicule, excepting its head portion, is enclosed by well developed transparent alae, which are seen to advantage when the spicules project through the cloacal aperture.

This species has been recorded in India from the red-billed magpie, *Urocissa erythrorhynca occipitalis* by Baylis and Daubney (1922) and

from the red-billed chough, *Pyrrhocorax pyrrhocorax*, by Maplestone (1931). The latter author also described a form *Acuaria scutata* (1931) from an Indian tree-pie, *Dendrocitta rufa*. Maplestone states that this species while being "almost identical with *Acuaria anthuris*" differs



Acuaria anthuris.

Fig. 1.—Anterior end, lateral view. Fig. 2.—End-on view of head. Fig. 3.—Head, ventral view. Fig. 4.—Posterior end, male, ventral view. Fig. 5.—Posterior end, female, lateral view. Fig. 6.—Oesophagus. Fig. 7.—Vagina and common trunk of the uterus. Fig. 8.—Egg. Fig. 9.—Tip of the tail, female, ventral view. Fig. 10.—Pseudo-cordon." Fig. 11.—Cordon.

from it mainly in the presence of an accessory pair of cordons. Baylis (1939) regards *A. scutata* as identical with *A. anthuris* and draws attention to observations made by Williams (1929), who has pointed out that accessory cordons or "pseudo-cordons" as he calls them, are present in all species of the genus *Acuaria*, "although differing considerably in

degree of development". The writer too has found that the presence of "pseudo-cordons" is a feature common to all species of *Acuaria*. The measurements given by Maplestone for *A. scutata* do not differ to any appreciable extent from those recorded herein for *A. anthuris*, and as such, the writer agrees with Baylis in regarding *A. scutata* as synonym of *A. anthuris*.

Host: *Corvus splendens* and *C. macrorhynchus*.

Habitat: Gizzard.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Acuaria skrjabini Ozerskaĭ, 1926.

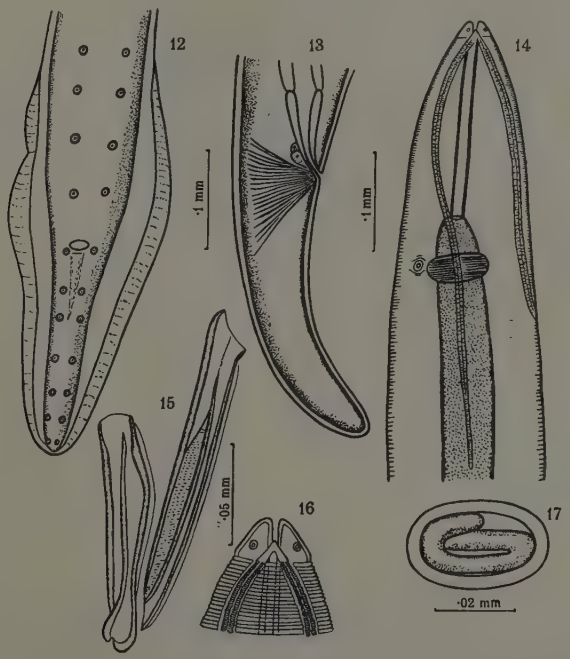
Syn., *Cheilospirura skrjabini* (Ozerskaĭ, 1926) Cram, 1927.

Out of seven specimens of house-sparrow (*Passer domesticus*) examined, one harboured a male and a female worm belonging to the species *Acuaria skrjabini*. These worms were recovered from the gizzard in which they were found lying under the horny layer.

The body in both sexes is of uniform diameter and is attenuated only near the anterior and the posterior ends. The female is considerably larger than the male, being about four times as long as the latter. The cuticle bears fine transverse striations, which at about the middle of the body are placed at intervals of 4μ in the male and 8μ in the female. The mouth is bounded by a pair of lateral lips which bear two pairs of submedian and one pair of lateral papillae. The cordons are short and faintly developed; they do not extend beyond the middle of the muscular portion of the oesophagus. The "pseudo-cordons" appear as irregular interruptions of the transverse striae at the lateral lines and extend forwardly as far as the anterior half of the pharynx. The cervical papillae are located at the level of the nerve-ring, which surrounds the muscular portion of the oesophagus near its junction with the pharynx. The excretory pore could not be observed even after prolonged search. The mouth leads into a long cylindrical pharynx which is followed by an oesophagus differentiated into two portions, a short muscular anterior and a long glandular posterior portion.

Female: The female measured 25.57 mm. in length and .285 mm. in maximum transverse diameter. The cordons extend .437 mm. from the head-end and occupy about 1/58 of the body length. Each cordon is longitudinally split in two but the dividing cleft disappears slightly in front of the termination of the cordon. The cervical papillae are .248

mm. from the head end. The pharynx is .188 mm. long and the constituent portions of the oesophagus are .74 and 1.26 mm. long respectively. The total length of the oesophagus forms roughly 1/13 of the body length. The tail, which is bluntly pointed is .308 mm. long. The vulva, which appears like a transverse slit and is flush with the body surface, is placed at a distance of 12.8 mm. from the anterior end



Acuaria skrjabini.

Fig. 12.—Posterior end, male, ventral view. Fig. 13.—Posterior end, female, lateral view. Fig. 14.—Anterior end, female, subventral view. Fig. 15.—Spicules, ventral view. Fig. 16.—Head end, female, ventral view. Fig. 17.—Egg.

and is thus located at about the middle of the body. The vagina runs posteriorly from the vulva and leads into the common trunk of the uterus. The genitalia are amphidelphic in arrangement. The eggs are thick shelled and embryonated at the time of laying. Those found in the common trunk of the uterus measured 37μ long by 23μ wide.

Male: The male measured 5.5 mm. in length and .154 mm. in maximum transverse diameter. The cordons extend 177μ from the head end and occupy 1/31 of the body length. The pharynx is 111μ long and the two portions of the oesophagus .286 and .72 mm. long respectively. The total length of the latter forms about 1/5.5 of the body length. The tail is .196 mm. long and is provided with a pair of lateral alae. The caudal papillae are very small, there being altogether 11 pairs. Out of these, four pairs are pre-anal, one pair ad-anal, and the remaining six pairs post-anal in position. The two spicules are .163 and .121 mm. long respectively. Both are grooved and while the left one is of uniform diameter and has a pointed tip, the right one is broader in its anterior half and has a rounded and notched tip.

This species was described by Ozerskaĭ (1926) from the house-sparrow (Don district, Russia). It is here recorded from the same host in the Hyderabad State. The chief differences are that the male is much smaller and the caudal papillae number 11 pairs instead of 10 pairs as described by Ozerskaĭ. The differences of measurements, however, are very minute and do not call for special mention. This species was assigned to the genus *Cheilospirura* by Cram (1926), but Williams (1929) replaced it in the genus *Acuaria* presumably on the ground that the spicules in this species are subequal in length and not markedly unequal as in *Cheilospirura*.

Host: Passer domesticus.

Habitat: Gizzard.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Acuaria kungi n.sp.

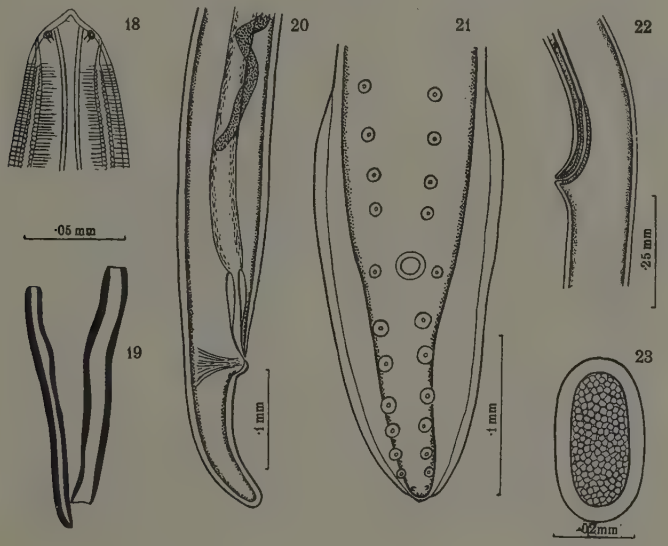
Three specimens of this species, two males and a female lacking the head-end, were collected from a "brown-backed Indian Robin", *Saxicoloides fulicata.* The worms occurred under the corneous layer of the gizzard.

These are rather slender-bodied worms, with a thin cuticle bearing fine transverse striations. The head is 31μ wide and bears two triangular lateral lips. The cordons originate in pairs between the bases of the lips and extend backwardly for a distance of .226 mm., just posterior to the level of the nerve-ring. They do not recurve or anastomose posteriorly. Each is divided longitudinally into right and left halves by a median groove.

The head bears two pairs of sub-median papillae and the mouth

opens into a cylindrical pharynx. The latter leads posteriorly into a long oesophagus which is partly muscular and partly glandular. The nerve-ring surrounds the muscular portion of the oesophagus near its junction with the pharynx.

The solitary female specimen available lacks the head-end. It measured 15.7 mm. in length with a transverse diameter of .179 mm. at the level of the vulva. The tail is short and bluntly rounded; it is



Acuaria kungi n.sp.
Fig. 18.—Head end, male, lateral view. Fig. 19.—Spicules, ventral view. Fig. 20.—Posterior end, female, lateral view. Fig. 21.—Posterior end, male, ventral view. Fig. 22.—Body of female, showing vulva and vagina. Fig. 23.—Egg.

.178 mm. long. The vulva is located on a prominent projection of the body wall and is 8.571 mm. from the tip of the tail. The vagina runs anteriorly from the vulva and is followed by the common trunk of the uterus. The eggs are thick-shelled and oblong in shape; they are embryonated at the time of laying and are 40μ long by 23μ wide.

Male: The male measures 8.25 mm. long and has a maximum width of 112μ at about the middle of the body. The pharynx is 164μ long and the length measurements of the two portions of oesophagus are .359 and 1.097 mm. The two portions together constitute about 1/5.6 of the

body length. The nerve-ring is .2 mm. from the head-end. The tail is narrowed posterior to the cloaca. It forms about 1/59 of the body length. It is provided with a pair of lateral papillae, 262μ long. There are altogether 12 pairs of caudal papillae, out of which four pairs are pre-anal, one ad-anal, and seven post-anal in position. The spicules are almost equal in length and measure 119μ and 122μ respectively. The right spicule is thinner and pointed at its tip, whereas the left spicule is relatively stouter and has a broad tip.

This species bears resemblance to *Acuaria papillifera* (Linstow, 1878) in possessing 12 pairs of somewhat similarly disposed prominent caudal papillae but differs from it markedly in size, being almost double in length and in the fact that the spicules are almost equal in length. *A. tenuis* (Dujardin, 1845) from an allied host *Saxicola rubetra,* which is rather insufficiently known, differs in length measurements of the body, oesophageal portions, pharynx, and also in the proportionate length of the tail and caudal alae. The present species also has affinities with *A. attenuata* (Rudolphi, 1819) but differs from it in length of the oesophagus. While *A. attenuata* has thick and sinuous cordons, the new species is characterised by the presence of straight and rather narrow ones. It is proposed to name the species *Acuaria kungi* after my colleague Mr. C. C. Kung.

Host: Saxicoloides fulicata.

Habitat: Gizzard.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

The type specimens will be deposited in the Department of Parasitology, London School of Hygiene and Tropical Medicine.

Acuaria sp.

A single male specimen of this form was collected from the gizzard of a rose-coloured pastor, *Pastor roseus*. It measured 6.68 mm. in length. The head is sub-triangular with a pointed apex and somewhat rounded sides. The pharynx is 163μ long and is followed by a divided oesophagus, the two constituent portions of which are .396 and .98 mm. long respectively. The cordons are rather faintly developed and extend .247 mm. from the head-end. The tail is .163 mm. long and is provided with a pair of lateral alae. There are altogether 10 pairs of caudal papillae, out of which four are pre-anal and the remaining six post-anal in position. The spicules are 112 and 162μ long respectively and both are grooved and also have pointed tips. Due to the lack of sufficient material

the species could not be determined.

Host: Pastor roseus.

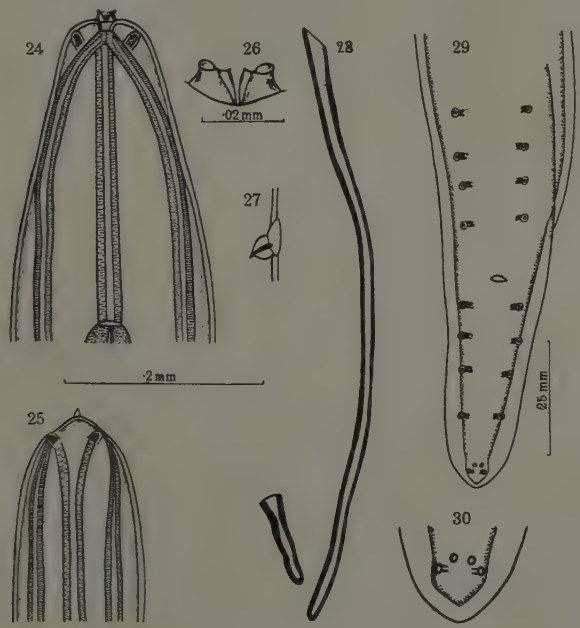
Habitat: Gizzard.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

CHEILOSPIRURA (DIESING, 1851).

Cheilospirura hyderabadensis n.sp.

A single male specimen of this species was recovered once from the oesophagus of a Sparrow-Hawk (*Accipiter nisus*). The worm measures 15.72 mm. in length and has a maximum transverse diameter of .428 mm. The body is cylindrical in shape and attenuated towards the extremities. The cuticle bears transverse striations which are much finer and less prominent in the head region; they are separated by



Cheilospirura hyderabadensis, n.sp.

Fig. 24.—Anterior end, male, ventral view. Fig. 25.—Head, lateral view. Fig. 26.—Teeth with spiny crests, ventral view. Fig. 27.—Cervical papilla. Fig. 28.—Spicules, ventral view. Fig. 29.—Posterior end, male, ventral view. Fig. 30.—Tip of the tail, male, ventral view.

intervals of 5μ in the middle of the body while they are only 4μ apart near the anterior end. The cervical papillae are quite conspicuous structures, leaf-like in appearance and directed obliquely backwards; they are 470μ from the anterior end and are located posterior to the junction of the buccal cavity and the oesophagus. The head is broadly rounded and bears two pairs of well developed pedunculated papillae. The mouth is bounded by a pair of laterally situated lips each bearing a prominent tooth. The teeth project forwardly between the lips and each possesses a spiny crest on its inner margin. The cordons are in the form of four cuticular ridges starting in pairs between the lips. As they proceed backwardly they diverge from each other and extend along the submedian zones of the body wall; they are 2.54 mm. long and occupy the anterior two-fifths of the oesophagus. The posterior ends of the cordons do not recurve or anatomose with each other. A longitudinal median groove running along the entire length of the cordon splits it into two halves. When seen dorso-ventrally the external half presents a striated appearance while the other half is without any striae.

The mouth leads into a relatively long pharynx having thick and transversely striated walls. It measures .304 mm. in length and its anterior end presents a funnel-shaped appearance in lateral view. It is followed by a cylindrical oesophagus 5.47 mm. long. There is a single testis extending almost to the origin of the intestine where it terminates in a posteriorly directed tip. The tail is flexed ventrally and has narrow caudal alae. The cloacal aperture is .461 mm. from the tip of the tail. The caudal papillae are pedunculated and arranged symmetrically on the ventral surface of the tail. Of these the pre-anals are arranged in four pairs which are equally spaced. Posterior to the cloaca there are four pairs of post-anal papillae, then near the tip of the tail there is an additional pair of pedunculated papillae, whilst immediately in front of this and more ventrally situated is a pair of sessile papillae. The spicules are extremely unequal in length and dissimilar in shape; both are pointed towards their tips and have slightly expanded heads. Whilst the long spicule has an almost uniform diameter throughout its length, the short one has an uneven contour; they measure 614 and 95μ respectively and the ratio between their lengths is roughly 6 : 1.

Discussion.

The genus *Cheilospirura* was created by Diesing in 1861 with *Cheilospirura hamulosa* (Diesing, 1851) as its type. *C. hamulosa* and *C.*

pavonis Ortlepp, 1925 differ from all the other species in the great length of the cordons which extend posterior to the middle of the body. In the present species and the other species to be considered they are confined to the oesophageal region. The species *C. spinosa* Cram, 1927, *C. multispinosa* Vigueros, 1938, *C. centrocerci* Simon, 1939, are distinguishable from the rest by the characteristic spiny nature of their cordons. Again *C. rotundata* (Linstow, 1907) has only five pairs of post-anal papillae in the male, while the new species and the remaining three species to be considered all possess six pairs of post-anal papillae. On the basis of the spicule lengths these species could be distinguished from one another; in *C. falconis* Clapham, 1947, the ratio is 1 : 2, in *C. gruveli* (Gendre, 1913) it is 1 : 3, and in the new species it is 1 : 6. The cervical papillae also differ in *C. falconis* and the new species; they are hook-like and pointed anteriorly in the former while leaf-shaped and directed obliquely backwards in the latter. The cordons in these two species, though of equal lengths, run in a straight course in the new species whereas they are wavy in *C. falconis*.

Host: Accipiter nisus.

Habitat: Oesophagus.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

The type specimen will be deposited in the Department of Parasitology, London School of Hygiene and Tropical Medicine.

SYNHIMANTUS Railliet, Henry and Sisoff, 1912.

Synhimantus laticeps (Rudolphi, 1819) Stiles and Hassall, 1920.

Three specimens of this species, one male and two females, were recovered once from an Asiatic sparrow-hawk, *Accipiter nisus*. As the worms were firmly attached to the mucous lining of the proventriculus, great care had to be taken to detach them without any injury to the head.

In both sexes the body is of uniform diameter whilst the anterior extremity is slightly narrowed. The cuticle is thick and bears transverse striations which are 14μ and 23μ apart in the male and female respectively. The head is rounded and bears two pairs of sub-median papillae, which are very prominent. The mouth is guarded by a pair of lateral lips, each of which bears an anteriorly directed tooth. The cordons originate in pairs between the bases of the lips and their inner margins are scalloped. After running posteriorly for a distance of .5 mm. in the

female and .379 mm. in the male they loop forwardly to run in an anterior direction and unite in pairs across the lateral fields, the transverse anastomosis lying 182μ from the head end.

The cervical papillae are posterior to the cordons and are tricuspid. They are .571 mm. from the tip of the head in the female and .612 mm. in the male. The excretory pore in the latter is 433μ from the head end. The mouth leads into a narrow cylindrical pharynx measuring .256 mm. The oesophagus is composed of an anterior muscular and a posterior glandular portion. The two portions measure .96 mm. and 3.16 mm. in the female and .865 and 2.8 mm. in the male respectively.

Female: The female is considerably larger than the male and has a length of 14 mm. with a maximum width of 466μ. The vulva is situated at about the middle of the body. The vagina runs obliquely backwards from the vulva and is connected to a muscular ovejector which has a slightly developed sphinctre. The tail is short and bluntly pointed measuring 203μ in length. The eggs which are 38μ long by 22μ wide, contain an embryo at the time of oviposition.

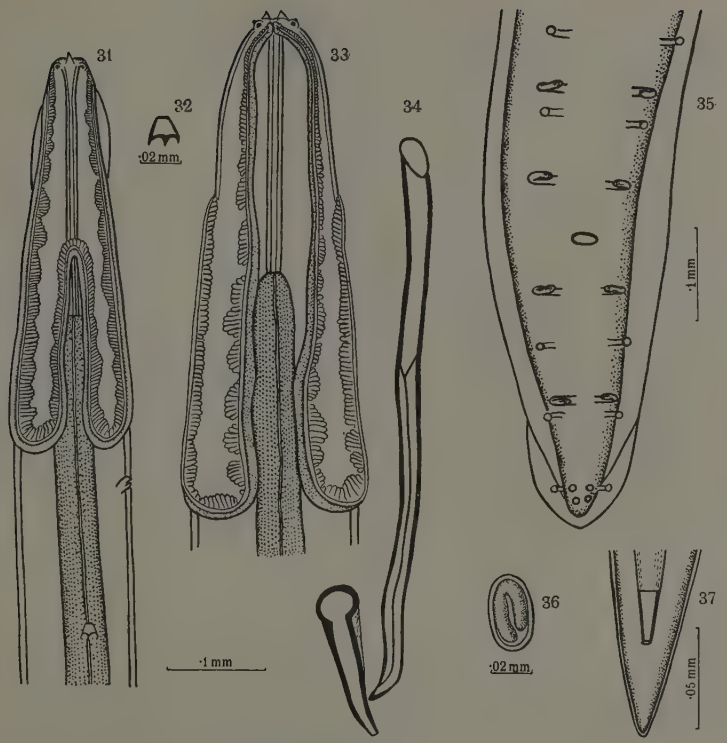
Male: The male is smaller and considerably thinner than the female. It measures 9.6 mm. in length and .238 mm. in greatest width. The tail is bluntly pointed and provided with a pair of lateral alae. There are four pairs of pre-anal and five pairs of post-anal pedunculated papillae. One remarkable feature about the papillae is that the second and fourth pairs of pre-anals and the first and third pairs of post-anals are very long as compared with the remaining pairs and form loops. Seurat (1916) also figured these papillae as being looped. Behind the last pair and the tip of the tail there are two pairs of very small sessile papillae which have not been noted by previous authors. The spicules are extremely unequal in length and dissimilar in shape. The ratio between their lengths is 1 : 3.5. The left spicule measures 158μ and the right 575μ in length. Whilst the latter has a uniform diameter the former has a broad anterior portion tapering gradually towards the tip.

This species is recorded here for the first time from Hyderabad and is also a new record for India.

Host: Accipiter nisus.

Habitat: Proventriculus.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).



Synhimantus laticeps.

Fig. 31.—Anterior end, male, lateral view. Fig. 32.—Cervical papilla. Fig. 33.—Anterior end, female, ventral view. Fig. 34.—Spicules, ventral view. Fig. 35.—Posterior end, male, ventral view. Fig. 36.—Egg. Fig. 37.—Posterior end, female, ventral view.

Dispharynx Raillet, Henry, and Sisoff, 1912.

Dispharynx spiralis (Molin, 1858) Skrjabin, 1916.

Numerous males and females of this species were collected twice from the oesophagus and the proventriculus of the jungle-crow, *Corvus macrorhynchus*. The heads of the worms were deeply embedded in the mucous lining and hence had to be detached very carefully. Up to now this species has been recorded from various birds, chiefly galliform birds.

The writer records it for the first time from corvidae. As it has previously been described in detail by Baylis (1939) and others, only a brief description of the parasite is given below.

Female: 4-5.45 mm. long by .44-.5 mm. wide, relatively much stouter than the male. Pharynx .126 mm. long, anterior oesophagus .481 mm. and posterior oesophagus .87 mm. long. Bicuspid cervical papillae in front of the posterior limits of the cordons. Vulva in the posterior third of the body, 1.21 mm. from the tip of the tail. Anus 147μ from the tail end. Eggs 37μ by 19μ embryonated at the time of laying.

Male: Slender, 3.75 to 6.62 mm. long with a maximum width of 224-312μ. Cordons extending 233-420μ from head end. The bicuspid papillae situated between the cordons, just in front of their posterior limits. The pharynx measures 93-107μ in length. The anterior portion of the oesophagus is about .4-.88 mm. long and its posterior portion about 1.46-1.84 mm. Spicules 165 and 482μ long respectively. Caudal papillae pedunculated, four pairs pre-anal and five pairs post-anal. Tail 390μ long, strongly flexed ventrally.

Host: Corvus macrorhynchus.

Habitat: Oesophagus and proventriculus.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

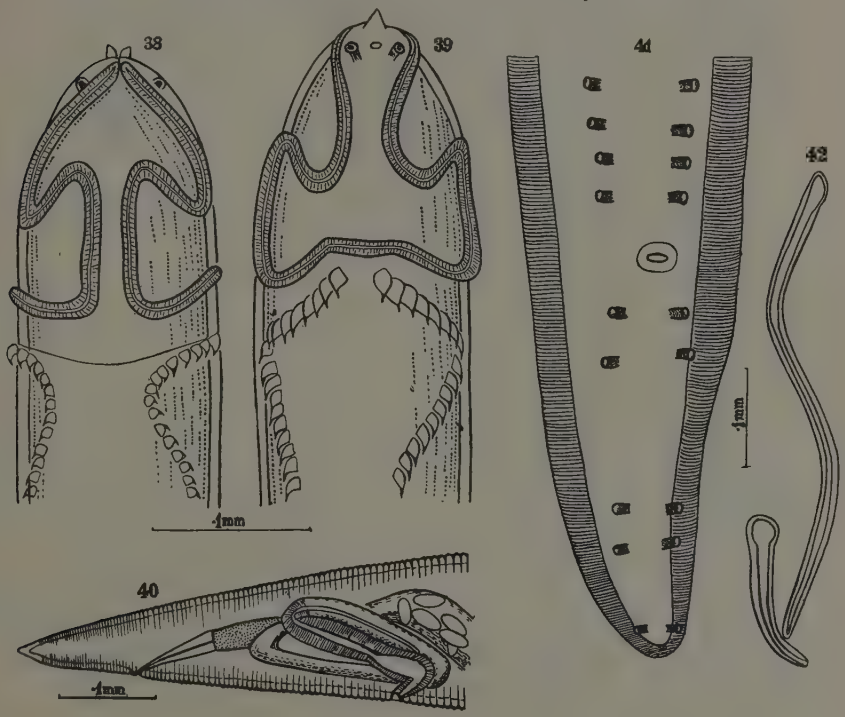
ECHINURIA Soloviev, 1912.

Echinuria horrida (Rudolphi, 1809) Cram, 1927.

On one occasion two female specimens of this species, one complete and one fragment, were collected from the white-breasted kingfisher, *Halcyon smyrnensis*. The worms were recovered from the oral cavity in which they were found firmly attached to the mucous membrane. The body is of uniform diameter except that it narrows from a little in front of the vulva to form a short and conical tail. The head presents a dome-shaped appearance and is ornamented with cordons which originate in pairs from the base of the laterally situated lips. They extend posteriorly about 166μ from the head-end and anastomose in pairs in the mid-lateral line of the body. The united cordons enclose an area which could be differentiated in three parts, an anterior slightly expanded hood-like structure separated by a narrow neck from a broad base with two laterally extended loops.

Immediately posterior to the cordons there is a transverse groove which is quite prominent in the dorso-ventral aspect of the head, and immediately posterior to this there are two rows of spines. At first the

two rows start quite close together in the lateral fields and as they proceed backwardly they diverge in an arc-like manner and then run parallel to the posterior end of the body. The cuticle bears transverse striae which are 9μ apart in the middle of the body. The head carries



Echinuria horrida.

Fig. 38.—Anterior end, female, ventral view. Fig. 39.—Anterior end, female, lateral view. Fig. 40.—Posterior end, female, lateral view.

Dispharynx spiralis.

Fig. 41.—Posterior end, male, ventral view. Fig. 42.—Spicules, ventral view.

two pairs of submedian papillae and a pair of laterally situated "amphids" or lateral papillae. The mouth which is bounded by two small lateral lips projecting in the form of two triangular lobes in front, opens into a narrow cylindrical pharynx measuring .349 mm. in length. Its length as compared with the body length is in the ratio of 1 :27.

The cylindrical oesophagus is composed of two parts which together measure 3.42 mm. The anal aperture is located 127μ from the tip of the tail and the vulva lies 258μ anterior to it. The vagina runs obliquely in front and is then joined to a posteriorly directed muscular ovejector which is differentiated into an anterior expanded pars ejectrix and a tubular pars haustrix. The latter is joined to the common trunk of the uterus which runs forward to the level of the vulva, then back as far as the posterior bend of the ovejector where it turns forward once again. The eggs measured 33μ long by 16μ wide. They were thin-shelled and unsegmented.

Up to now this species has been reported from Europe in *Scolopax gallinula, Charadrius histicula* and *Tringa* spp. It is here reported for the first time from India and also from a new host.

Host: Halcyon smyrnensis.

Habitat: Mouth cavity.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

RUSGUNIELLA Seurat, 1919.

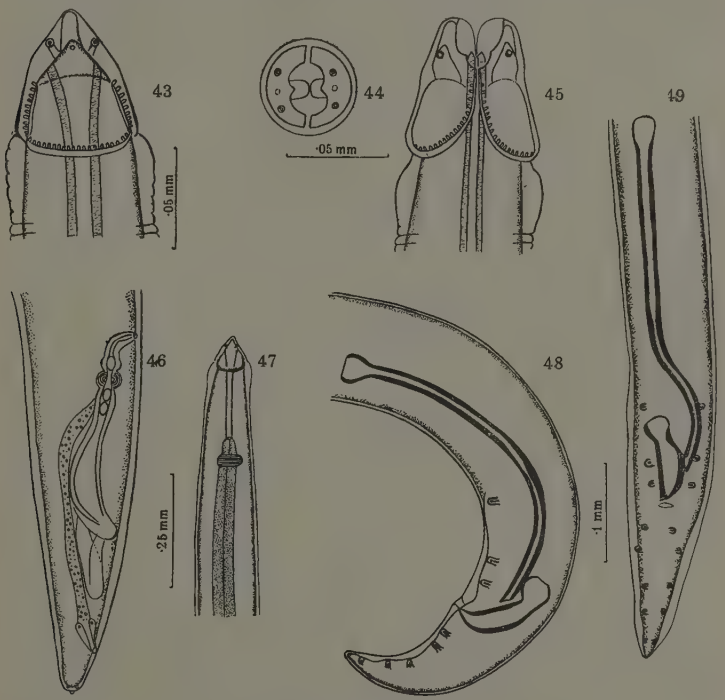
Rusguniella brevis Maplestone, 1931.

This worm was recovered twice from the gizzard of the white-breasted king-fisher, *Halcyon smyrnensis*. A total of four specimens was collected, two males and two females.

These are long and slender-bodied worms, the females being considerably larger than the males. In both sexes the body is attenuated towards the anterior end. The cuticle is transversely striated, the striations being more pronounced in the middle region of the body. The head is dome-shaped and is distinctly set off from the rest of the body and its cuticle is slightly inflated. The cordons are in the form of two short crescents, starting dorso-ventrally between bases of the lips and extending backwardly round the lateral surface of the head. They are frill-like structures with a denticulated inner margin. Immediately behind the cordons there is a circular groove which is quite prominent in the dorso-ventral view of the head. The nerve-ring encircles the anterior portion of the oesophagus near its junction with the pharynx.

The mouth is bounded by two lateral lips, from which two transparent cuticular projections extend inwardly to meet in the middle. (Fig. 44.) Each lip bears a pair of pedunculated sub-median cephalic papillae with a laterally situated amphid lying between them. The mouth leads into a long cylindrical pharynx which is heavily chitinized and marked with fine transverse striae. Its anterior end is slightly expanded in lateral aspect.

Female: The females, which are two to three times as large as the males, vary in length from 11.94 to 18.56 mm., with a maximum width of .333 to .38 mm. The cordons extend 73μ from the head-end. The pharynx is .204 mm. long and the total length of the oesophagus is 1.105 mm. The anal-opening is 0.112 mm. from the tip of the tail



Rusguniella brevis.

Fig. 43.—Head, lateral view. Fig. 44.—End-on view of head. Fig. 45.—Head, ventral view. Fig. 46.—Posterior end, female, lateral view. Fig. 47.—Anterior end, lateral view. Fig. 48.—Posterior end, male, lateral view. Fig. 49.—Posterior end, male, ventral view.

and the vulva, which is flush with the general surface of the body, is .689 mm. anterior to it. There is a pair of small papillae near the tip of the tail. The vagina runs posteriorly from the vulva and is joined to an ovejector which has a distinct sphincter and a long sac-like reservoir. The latter gives origin to two uteri which immediately turn forward and run parallel anteriorly. The filiform ovaries are coiled

tubules located near the anterior end of the intestine and extending slightly into the oesophageal region. The eggs are thick shelled and those found in the ovejector measured 31μ long by 17μ wide.

Male: The male measured 5.714-5.856 mm. in length, reaching a maximum width of .105 mm. near the middle of the body. The cordons extend 42μ from the head end and the pharynx is .142 mm. long. The total length of the oesophagus is 1.82 mm. of which .46 mm. is occupied by the muscular portion and 1.365 mm. by the glandular portion. The nerve-ring is 170μ from the anterior end. The tail is flexed ventrally and the cloacal aperture lies at a distance of .156 mm. from the tip of the tail. The caudal alae are .276 mm. long. There are eight pairs of caudal papillae, three pairs being pre-anal and the remaining five pairs post-anal in position. As regards the pre-anals the distance between the first and second pairs is almost double that between the second and third pairs. The post-anal papillae form two groups. The anterior group consists of the first and second post-anals and the posterior group is comprised of the third, fourth and fifth pairs. All the papillae described above are pedunculated. The spicules are unequal and dissimilar in shape. The right spicule, which is short and thick, has an expanded head and a beak-like pointed tip. The left spicule is relatively much more slender and is between 3-4 times as large as the right one. It has a distinct expanded head, sharply marked off from the body of the spicule. The length measurements of the two spicules are 89 to 104μ and 371-373μ respectively.

Discussion.

This species was described by Maplestone (1931) from the gizzard of *Ceryle alcyon*. The structure of the head figured by him does not exactly correspond with that observed by the author, in the fact that rounded cuticular projections of the lips were not represented in the dorso-ventral view of the head. The cuticle of the head is more distinctly inflated in the species described herein and the denticulations of the cordons are larger in size and fewer in number. In the male an additional pair of pre-anal papillae has been noted by the author. In the female the ovejector has been observed to possess a distinct sphincter. Wehr (1934) regards *Rusguniella brevis* as synonym of *Aviculariella alcyona* Wehr, 1931. But the author agrees with Baylis (1939) who considers that these are distinct species. The main difference between these two species is that the cuticular inflation in *Avicularielia alcyona* is very prominent, whereas in *R. brevis* it is less evident, although the writer

had the opportunity of examining the worms in live condition. Moreover the distinct forward bend of the cordon at its middle as figured by Wehr is absent in the Indian species and the cordons extend relatively farther backwards.

Host: Halcyon smyrnensis.

Habitat: Gizzard.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

Summary.

1. *Acuaria anthuris,* the type species of the genus *Acuaria,* is described in detail and *A. scutata* is considered to be synonymous with it.

2. *Acuaria skrjabini* from *Passer domesticus* has been described and compared with the original description. It is a new record for India and Hyderabad.

3. *Acuaria kungi* n.sp. from *Saxicoloides fulicata* is described and compared with related species.

4. *Acuaria* sp. is recorded from *Pastor roseus.*

5. A new species of *Cheilospirura* from *Accipiter nisus* is described and compared with related species.

6. *Synhimantus laticeps* from *Accipiter nisus* is described. It is a new record for India and Hyderabad.

7. *Dispharynx spiralis* is reported for the first time from corvidae.

8. *Echinuria horrida* is recorded from a new host, *Halcyon smyrnensis.*

9. *Rusguniella brevis* from *Halcyon smyrnensis* is described in detail and compared with *Aviculariella alcyona.*

Acknowledgments.

The writer takes this opportunity of expressing his deep gratitude to Professor J. J. C. Buckley under whose guidance the entire work was carried out. Thanks are due to Dr. P. L. LeRoux for criticism and suggestions. The writer is indebted to Professor H. E. Shortt, Director of the Department of Parasitology, for providing facilities, and to the members of the staff, especially Mr. F. R. N. Pester, for help rendered during the progress of the work.

References

Baylis, H. A., 1939.—The Fauna of British India, including Ceylon and Burma. *Nematoda. Vol. II., London.*

Chitwood, B. G., and Wehr, E. E., 1934.—" The value of cephalic structures as characters in nematode classification, with special reference to the superfamily Spiruroidea." *Z. Parasitenk,* 7 (3), 273–335. (*W.L.* 23536a.)

Clapham, P. A., 1947.—" On *Vianella africana* n.sp., *Cheilospirura falconis* n.sp., and *Oesophagostomum tridentatum* Maplestone, 1932." *J. Helminth*, XXII (1), 29–36. (*W.L.* 11224b.)

Cram, Eloise B., 1927.—" Bird Parasites of the Nematode Suborders Strongylata, Ascaridata, and Spirurata." *Bull. U.S. nat.* Mus., no. 140. (*W.L.* 5659.)

Gendre, E., 1913.—" Sur une espéce de Dispharage peu connue." *P.V. Soc. Linn. Bordeaux*, April, 1913. (*W.L.* 16996.)

Linstow, O. Von., 1878.—" Neue Beobachtungen an Helminthen." *Arch. Naturgesch.* (*W.L.* 1782.)

Maplestone, P. A., 1931.—" Parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens.—parts 4–8. *Rec. Indian Mus.*, XXXIII, (4–8), 71–171. (*W.L.* 17746.)

1932.—" Parasitic Nematodes obtained from animals dying in the Calcutta Zoological Gardens." *Rec. Indian Mus.*, XXXIV, 229–261. (*W.L.* 17746.)

Ortlepp, J. R., 1925.—" On two nematode parasites from the gizzard of Pea-fowls." *J. Helminth*, III (5), 177–184. (*W.L.* 11224b.)

Railliet, A., Henry, A., and Sisoff, P., 1912.—" Sur les affinites des Dispharages (Acuaria Bremser), Nematodes Parasites des Oiseaux." *C. R. Soc. Biol. Paris*, 73, 622–624. (*W.L.* 6630.)

Seurat, L. G., 1915.—" Sur la Morphologie de l'*Acuaria laticeps* (Rud.)." *C. R. Soc. Biol. Paris*, 73, 41–44. (*W.L.* 6630.)

Simon, F., 1939.—*Cheilospirura centrocerci*, a new nematode from the sage grouse *Centrocercus urophasianus*." *Trans. Amer. Micr. Soc.*, LVIII 58 (1), 78–80. (*W. L.* 21400a.)

Vigueras, P. I., 1938.—" Nota sobre algunos nematodes parasitos nuevos de Cuba." *Livro Jubilar do Professor Lauro Travassos, Rio de Janeiro*,. 1938, XX, 589 pp.

Wehr, E. E., 1931.—" Description of a new genus and species of nematode worm occurring in the North-western belted king-fisher, with a key to the genera of the subfamily Acuariinae." *Proc. U.S. nat. Mus.*, 79, *Art.* 5, 1–4. (*W.L.* 16944.)

Wehr, E. E., 1934.—" *Rusguniella brevis* Maplestone, 1931, as a synonym of *Aviculariella alcyona* Wehr, 1931." *Proc. Helm. Soc. Washington*, 1 (1), 11–12.

Williams, O. L., 1929.—"A critical analysis of the specific characters of the genus *Acuaria*, Nematodes of Birds, with description of new American species." *Univ. Calif. Publ. Zool.* 33, 69–107. (*W.L.* 22084.)

Yorke, W. & Maplestone, P. A., 1926.—The Nematode Parasites of Vertebrates. *J. A. Churchill, London.*

Journal of Helminthology, Vol. XXII, No. 3/4, 1948. pp. 219—226.

**Gastronodus strasseni* Singh, 1934, and its affinities with *Spirocerca lupi* (Rudolphi, 1809) Chitwood, 1933.

By Satya Narayan Singh, M.Sc.

Reader-in-charge in Zoology, Osmania University, Hyderabad Deccan.

(From the Department of Parasitology, London School of Hygiene and Tropical Medicine.)

The writer in 1934 briefly described this nematode which was collected from the common musk-shrew at Hyderabad Deccan, creating for its reception the new genus and species *Gastronodus strasseni*. The following account deals with the anatomy of the worm and the close relationship which exists between the genera *Gastronodus* Singh and *Spirocerca* Railliet and Henry, 1911.

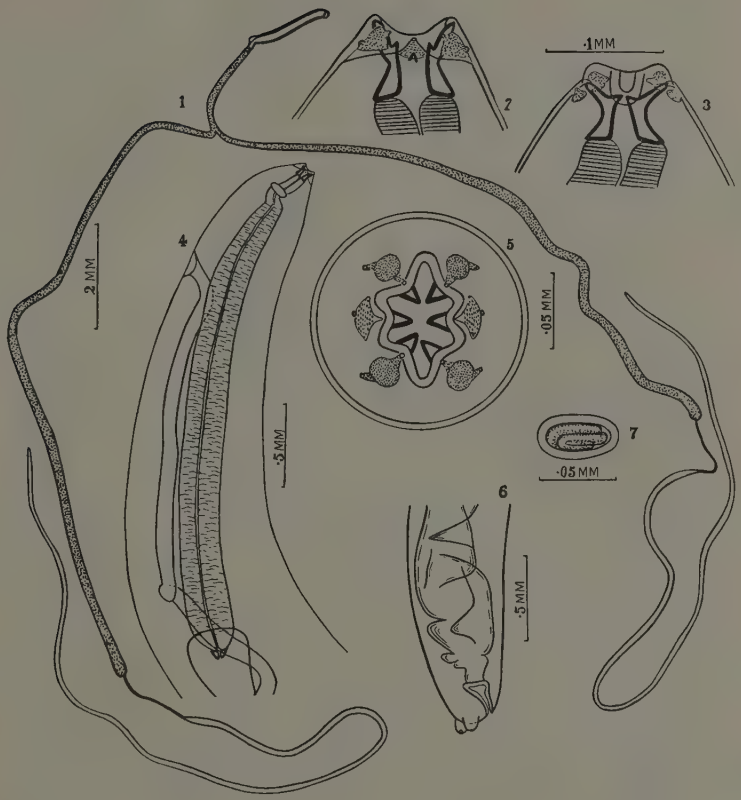
This worm is of common occurrence in the musk-shrew, *Crocidura caerulea*, at Hyderabad and it forms large nodules on the stomach walls of the host. In certain cases smaller nodules were also found in the mesentery. These are medium sized worms and when freshly extracted appear blood red in colour. The body is coiled and generally flexed at the extremities. In males and young adult females the body is of almost uniform diameter with blunt anterior and posterior ends, whereas in fully grown and gravid females the posterior 2/3 of the body is distinctly thicker than the anterior portion. The males are slender and smaller than the females. The cuticle is thick and bears fine transverse striations which are set quite close together. Irregular transverse wrinkles are also present on the body wall. The cervical papillae are located at the level of the nerve-ring and have the appearance of fine bristles.

The mouth opening is roughly hexagonal and is bounded by a sinuous cuticular band. Into this, project internal terminations from the cephalic papillae. These are well developed and comprise two pairs of submedian—one pair of subdorsal and one pair of subventral—and one pair of lateral papillae. The submedian papillae with the overlying cuticle are quite protuberant on the summit of the head, while the lateral papillae, sunk in the notches between them, are situated at a lower level, with the result that the head seems to possess a pair of bilobed lateral lips. The body of the submedian papilla is almost rounded

* *Castronodus strassenii* as it appeared in Current Science, vol. 2 (8) is a misprint of *Gastronodus strasseni* and hence was accordingly corrected in the subsequent issue of the journal.

and sends out an inner branch, already referred to, with a papillary termination. From the outer surface of the papilla, an external branch facing the lateral papilla is given out. The lateral papillae differ in shape from the median papillae being conical with lateral expansions. The external branches, corresponding to those present on the submedian papillae, are lacking whilst the inner terminations, though present, differ in character. These appear like conical projections apparently without papillary terminations. The mouth cavity leads into the chitinous, thick-walled pharynx. At its entrance spring six forwardly directed teeth, one opposite each cephalic papilla, slightly below the level of its inner branch. These are not rugosities of the pharyngeal wall as met with in *Spirocerca* but are distinct teeth. In optical section it can be seen that the sides of the pharynx diverge very widely towards the anterior end. The oesophagus is differentiated into a short muscular anterior and a long glandular posterior portion. The nerve-ring surrounds the muscular part in its posterior half. The glandular portion is club-shaped with the hind end embedded in the anterior termination of the intestine. The latter is thicker than the oesophagus and its walls, especially towards the posterior end, are much folded. It opens into the rectum, which in the case of the female communicates directly with the exterior at the anus.

Female: The female measures 25-40 mm. in length with a maximum transverse diameter of 1.15 mm. The cuticular striations are separated by intervals of 3μ near the posterior end. The pharynx is .05-.068 mm. The two portions of the oesophagus measure .25-.27 and 2.5-2.82 mm. respectively, and the ratio between their lengths is roughly 1 : 10. The anus is subterminal and there is a pair of lateral papillae projecting beyond the tip of the tail. The female genital organs consist of a pair of ovaries, oviducts, uteri, and the vagina. The ovaries are coiled tubes lying in the posterior 1/3 of the body. Each ovary opens by a short narrow duct, the oviduct, into the uterus. The uteri run parallel in a winding course ultimately uniting to form the common trunk. At about the junction of the trunk and the vagina there is a blind sac-like growth. This structure though easily detected in the young adult, is hardly discernible in the fully grown specimens, being indicated only by a thickening of the wall in the corresponding position. The vagina runs forward and opens on the ventral surface of the body near the anterior end. The distance of the vulva from the anterior end is .82-1.5 mm. The head is often bent dorsally. The eggs are long and thick-shelled



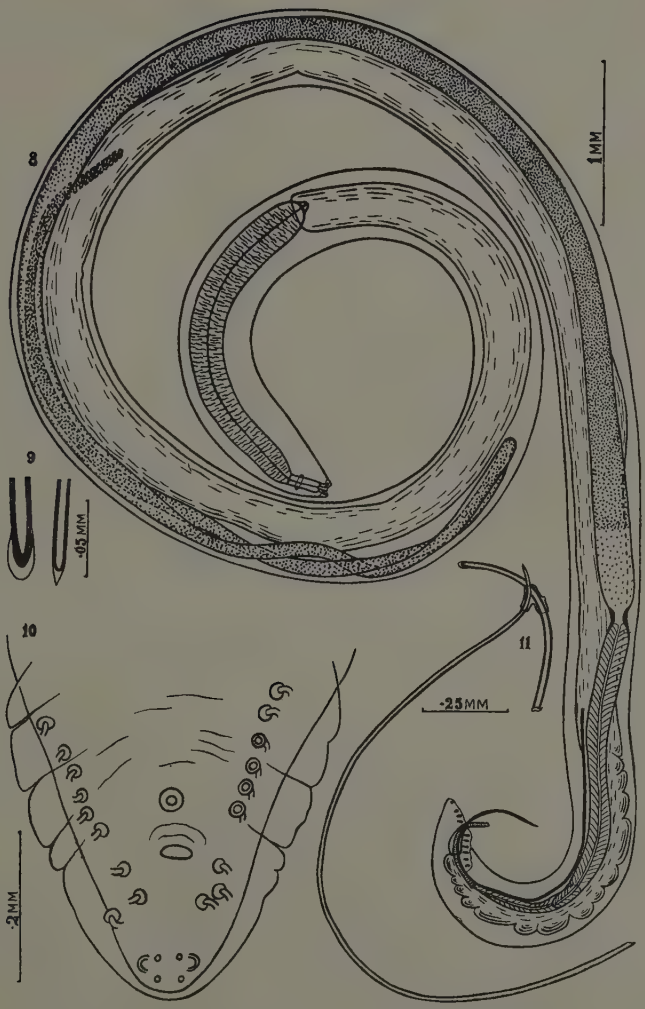
Gastronodus strasseni.

Fig. 1.—Reproductive organs of young adult female, with uteri and common trunk dotted, oviducts inked, and ovaries and vagina blank. Fig. 2.—Lateral view of head, showing cephalic papillae—two sub-median and one lateral—three teeth, pharynx and origin of oesophagus. Fig. 3—Ventral view of head, showing cephalic papillae —two sub-median and two lateral—two teeth, pharynx and origin of oesophagus. Fig. 4.—Anterior end of female in lateral view, showing oesophagus and vagina. Fig. 5.—End-on view of head, showing the mouth opening with six pharyngeal teeth, and two pairs of sub-median and one pair of lateral papillae. Fig. 6.—Posterior end of female, showing much folded intestine, rectum, and a pair of terminal papillae. Fig. 7.—Egg with embryo.

and contain a much coiled embryo at the time of laying; they measure 54μ by 30μ.

It was observed that the gravid females acquire an opening into the lumen of the stomach into which they project, evidently to discharge the eggs which are voided with the faeces of the host. In one instance the writer recovered a third stage larva in the partly digested ant-body which was found in the stomach of the shrew. It therefore appears probable that a local species of ant serves as an intermediate host. On two occasions it was further noted that encysted larvae occur on the inner surface of the body wall throughout the body cavity.

Male: The males vary in length from 16 to 26 mm. with a maximum transverse diameter of .56 mm. The cuticular striations are 3μ apart near the posterior end of the body. The pharynx is .05-.054 mm. in length and is roughly as long as it is broad. The muscular portion of the oesophagus measures .19-.22 mm. and the glandular portion 2.11-2.13 mm. The cervical papillae are .48 mm. from the anterior end. The tail is very short and rounded at the tip. It is provided with a pair of lateral alae which are rather narrow and have a tendency to form notches at the margins. The caudal papillae are fairly developed. The pre-anals comprise six pairs of equally spaced pedunculated papillae. Posterior to the cloacal aperture there is a group of three pairs of pedunculated papillae and near the tip of the tail a pair of large sessile papillae. A single large median papilla is situated anterior to the cloaca, and two pairs of minute papillae are located between the large sessile papillae at the tip of the tail. The spicules are distinctly unequal and dissimilar in shape; they frequently project outwardly through the cloacal opening. There is also present an accessory piece which is lightly chitinized and forms a v-shaped structure. Its two limbs, which are in the form of open grooves, act as guides for the spicules. The tip of the short spicule is enclosed by a broad and round ala while that which projects at the tip of the long spicule is conical and finely pointed. The two spicules are .45-.612 and 2.516-2.7 mm. long and their lengths are roughly in the proportion of 1 : 5. There is a single testis which extends forwardly into the vicinity of the anterior end of the intestine. Here it is recurved so as to form a twisted loop and then runs backwardly to end at about the middle of the body in a posteriorly pointed tip. At the other end it communicates with the seminal vesicle. The latter opens into the vas deferens, the junction between the two being marked by a distinct constriction. The vas deferens is a long duct with a glandular



Gastronodus strasseni.

Fig. 8.—Male in side view, showing general shape and structure. Fig. 9.—Tips of spicules. Fig. 10.—Posterior end, male, ventral view. Fig. 11.—Spicules with accessory piece.

epithelium and a narrow central lumen. At the posterior end it narrows to form a short ductus ejaculatorius which opens into the cloaca.

Discussion.

The genus *Gastronodus* was established by the writer in 1934 with *G. strasseni* as its type. The genus is closely allied to *Spirocerca* Railliet and Henry, 1911, the chief resemblances being the possession of six voluminous papillae having similar disposition round the mouth, the presence of a median pre-cloacal papilla in the male, and the similarity of the reproductive organs in the female. The two genera, however, differ from each other in the following features :—

1. In *Gastronodus* six teeth are present at the entrance to the pharynx. These are very prominent and project considerably into the middle of the oral cavity. In *Spirocerca,* however, they are absent, but in the corresponding position six rugosities of the pharyngeal wall are present, which as Baylis (1923) has pointed out, have been wrongly interpreted as "teeth" by Wedl (1856), and Railliet and Henry (1911). Baylis has shown that these teeth in *Spirocerca* are nothing more than indentations of the buccal capsule corresponding to the six internal branches from the main pulps of the cephalic papillae. The internal papillae are present in *Gastronodus* but the teeth described are set quite apart, at a lower level on the wall of the pharynx. Taking this feature alone into consideration *Gastronodus* appears to be more closely related to *Streptopharagus* Blanc, 1912 than to *Spirocerca* Railliet and Henry.

2. In the male of *Gastronodus* there are six pairs of pre-anal and four pairs of post-anal papillae excluding the minute papillae at the tip of the tail. In *Spirocerca,* however, there are only four pairs of pre-anal and two pairs of post-anal papillae.

3. The anus in the female of *Gastronodus* is subterminal whilst in *Spirocerca* a distinct tail, though short, is present.

In the light of recent observations it was considered advisable to give the following amended generic diagnosis :—

Medium-sized worms, somewhat stout bodied, with bluntly rounded anterior and posterior ends. Cuticle bearing fine transverse striations and marked with irregular transverse wrinkles. Distinct lips absent, but due to four projecting submedian papillae, head appears to possess bilobed lips. Mouth roughly hexagonal and bounded by a sinuous cuticularised band. Pharynx with thick chitinous walls and six teeth at its entrance. Oesophagus composed of a short muscular and a long

glandular portion. Intestine thicker than oesophagus. Cervical papillae bristle-like, located at the level of nerve-ring.

Female: Vulva in oesophageal region and generally sunk in a depression on the body wall. Anal aperture sub-terminal, with a pair of backwardly projecting lateral papillae at the tip of the tail. Eggs thick-shelled and embryonated at the time of laying.

Male: Posterior end strongly flexed ventrally. A pair of narrow caudal alae present. Caudal papiliae, six pairs pre-anal and four pairs post-anal. A median pre-cloacal papilla present. Spicules unequal and dissimilar, and provided with a lightly chitinized accessory piece.

Genotype.—*Gastronodus strasseni* Singh, 1934.

Parasitic in an insectivorous mammal, *Crocidura coerulea,* forming nodules on the stomach wall and less frequently in the mesentery.

Host: Crocidura coerulea.

Habitat: Nodules on the stomach wall.

Locality: Hyderabad Deccan (The Nizam's Dominions, India).

References.

Baylis, H. A., 1923.—" On the Nematode Genus *Streptopharagus* with some remarks on the Genus *Spirocerca.*" *Trans. R. Soc. Trop. Med. Hyg.* Vol. XVI (8), 486–497. (*W.L.* 21671.)

1939.—The Fauna of British India, including Ceylon and Burma. *Nematoda.* Vol. 2. London.

Baylis, H. A., and Daubney, R., 1926.—A Synopsis of the Families and Genera of Nematoda. *London* (*British Museum* (*Nat. Hist.*))

Railliet, A., and Henry, A., 1911.—" Helminthes du Porc recueillis par M. Bauche en Annam." *Bull. Soc. Path. exot.,* 4 (10), 693–699. (*W.L.* 5310.)

Singh, S. N., 1934.—" *Gastronodus strasseni,* g. et sp. n. A Nematode Parasite of *Crocidura coerulea*—the common Musk-shrew or, as it is usually called, Musk-rat (Vernacular : Chachundar)." *Curr. Sci.,* 2 (8), 287. (*W.L.* 7021d.)

Wedl, C., 1856.—" Uber die Mundwerkzeuge von Nematoden." *Z. B. Akad. Wiss. Wien.* (*W.L.* 20170.)

Yorke, W. & Maplestone, P. A., 1926.—The Nematode Parasites of Vertebrates. *J. A. Churchill, London.*

Index to Volume XXII

	PAGE
Acuaria anthuris, morphology of	199
synonymous with *Acuaria scutata*	199
kungi n.sp., in *Saxicoloides fulicata*	204
morphology of	204
madagascariensis n.sp., in *Dryolimnas cuvieri*	151
morphology of	151
skrjabini, morphology of	202
new record for India	202
Africa, occurrence of *Davainea madagascariensis* in	47
Anguillulina balsamophila, causing leaf galls	109
dipsaci, attacking field beans	1
oats	1
onions	1
parsnips	1
rhubarb	1
weeds	1
erythrinae, phasmids on male tale of	139
multicincta, phasmids on male tale of	139
robusta, phasmids on male tale of	139
Ardeola grayii, host of *Desmidocercella leiperi* n.sp.	77
Asymmetricostrongylus dissimilis, adults and encysted larvae of ..	93
in Tasmanian Wallaby ..	93
Avian Nematodes in Hyderabad State	77, 199
Bull-frog, host of *Camallanus mazabukae* n.sp.	141
Procamallanus brevis n.sp.	146
Camallanus mazabukae n.sp., in Bull-frog	141
morphology of	141
multilineatus n.sp., in *Rana catesbiana*	145
morphology of	145

PAGE

Cheilospirura falconis n.sp., in Kestrel 32
morphology of 32
hyderabadensis n.sp., in *Accipiter nisus* 207
morphology of 207
Coenurus of Man in England 73
Copsychus saularis, new host of *Oxyspirura popowi* 86
Cysticercus fasciolaris, development of 179
pisiformis, development of 179
transverse fission of 165
tenuicollis, endogenous budding in 63
Davainea madagascariensis in Africa 47
Definitive hosts of *Diphyllobothrium* spp. 16
Desmidocercella leiperi n.sp., in Indian Pond-heron 77
morphology of 78
Development of *Cysticercus fasciolaris* 179
pisiformis 179
Diphyllobothrium dendriticum in avian hosts 16
ditremum in avian hosts 16
epizootic in Ireland 13
spp., intermediate hosts of 14, 24
Dispharynx spiralis, new record from corvidae 211
Dryolimnas cuvieri, host of *Acuaria madagascariensis* 151
Echinuria horrida, morphology of 212
new record from *Halcyon smyrnensis* 212
Effects of D.-D. and soil sterilization on Potato-root Eelworm 117, 128
Endogenous budding in *Cysticercus tenuicollis* 63
England, a human case of spinal Coenurus in 73
incidence of *Trichinella spiralis* in 49
Falco tinnunculus, host of *Cheilospirura falconis* n.sp. 32
Field beans, hosts of *Anguillulina dipsaci* 1
Galloperdix spadicea, new host of *Oxyspirura mansoni* 84
Gastronodus strasseni, affinities with *Spirocerca lupi* 219
morphology of 219
Globocephaloides trifidospicularis n.sp., in Tasmanian Wallaby .. 94
morphology of 94

PAGE

Heterodera rostochiensis treated with D.-D. and soil sterilization 117, 128
Hyderabad State, avian Nematodes in 77, 199
Indian Robin, host of *Acuaria kungi* n.sp. 204
Intermediate hosts of *Diphyllobothrium* spp. 14, 24
Ireland, *Diphyllobothrium* spp. in 13
Kittacincla malabarica, host of *Oxyspirura indica* n.sp. 88
Labiostrongylus australis n.sp., in ? *Python spilotus* 102
morphology of 102
Macropus rufrogrisea fruticus, host of *Asymmetricostrongylus dissimilis* 93
Globocephaloides trifidospicularis n.sp. 94
Pharyngostrongylus longibursaris n.sp. 97
Zoniolaimus cobbi n.sp. 100
Oats, host of *Anguillulina dipsaci* 1
Oesophagostomum tridentatum, morphology of 34
Onions, host of *Anguillulina dipsaci* 1
Oxyspirura buccosulcatus n.sp. in Black-headed Myna 90
morphology of 90
indica n.sp., in Shama 88
morphology of 88
spp. from new hosts 84, 86
morphology of 84, 86
Parsnips, host of *Anguillulina dipsaci* 1
Phasmids in *Anguillulina* spp. 139
Physaloptera longibursaris n.sp., in Tasmanian Wallaby 97
morphology of 97
singhi n.sp., in ? Simian host 154
morphology of 154
Procamallanus brevis n.sp., in Bull-frog 146
morphology of 146
Rana catesbiana, host of *Camallanus multilineatus* n.sp. 145
Reptile hosts of *Tanqua gigantica* n.sp. 149
Rhubarb, host of *Anguillulina dipsaci* 1
Sparrow-hawk, host of *Cheilospirura hyderabadensis* n.sp. .. 207

	PAGE
Structure of leaf-galls caused by *Anguillulina balsamophila* ..	109
Synhimantus laticeps, morphology of	209
new record for India	209
Tanqua gigantica n.sp., in *Python reticulatus* and *Naja hannah* ..	149
morphology of	149
Technique for identifying Trichostrongyles	37
Temenuchus pagodarum, host of *Oxyspirura buccosulcata* n.sp. ..	90
Thryonomys swinderianus, host of *Vianella africana* n.sp. ..	29
Transverse fission of *Cysticercus pisiformis*	165
Trichinella spiralis, incidence in England	49
Trichostrongylus spp., technique for identifying	37
Vianella africana n.sp., in Cane rat	29
morphology of	29
Weeds, hosts of *Anguillulina dipsaci*	1
Zoniolaimus cobbi n.sp., in Tasmanian Wallaby	100
morphology of	100

Index of Authors

Clapham, P. A.	29, 37, 47
Crusz, H.	63, 73, 165, 179
Goodey, J. B.	109
Goodey, T.	1, 139
Hickey, M. D. and Harris, J. R.	13
Kung, C. C.	93, 141
Peters, B. G.	117, 128
Singh, S. N.	77, 199, 219
Young, M. R.	49

New Names in Volume XXII

New Species

	PAGE
ACUARIA KUNGI Singh, 1948	199
ANCYRACANTHOPSIS MADAGASCARIENSIS Kung, 1948	141
CAMALLANUS MAZABUKAE Kung, 1948	141
CAMALLANUS MULTILINEATUS Kung, 1948	141
CHEILOSPIRURA FALCONIS Clapham, 1947	29
CHEILOSPIRURA HYDERABADENSIS Singh, 1948 ..	199
DESMIDOCERCELLA LEIPERI Singh, 1948	77
GLOBOCEPHALOIDES TRIFIDOSPICULARIS Kung, 1948	93
LABIOSTRONGYLUS AUSTRALIS Kung, 1948	93
OXYSPIRURA BUCCOSULCATA Singh, 1948	77
OXYSPIRURA INDICA Singh, 1948	77
PHARYNGOSTRONGYLUS LONGIBURSARIS Kung, 1948	93
PHYSALOPTERA SINGHI Kung, 1948	141
PROCAMALLANUS BREVIS Kung, 1948	141
TANQUA GIGANTICA Kung, 1948	141
VIANELLA AFRICANA Clapham, 1947	29
ZONIOLAIMUS COBBI Kung, 1948	93

Erratum

Pp. 93-108—for "Australian Wallaby" read "Tasmanian Wallaby"